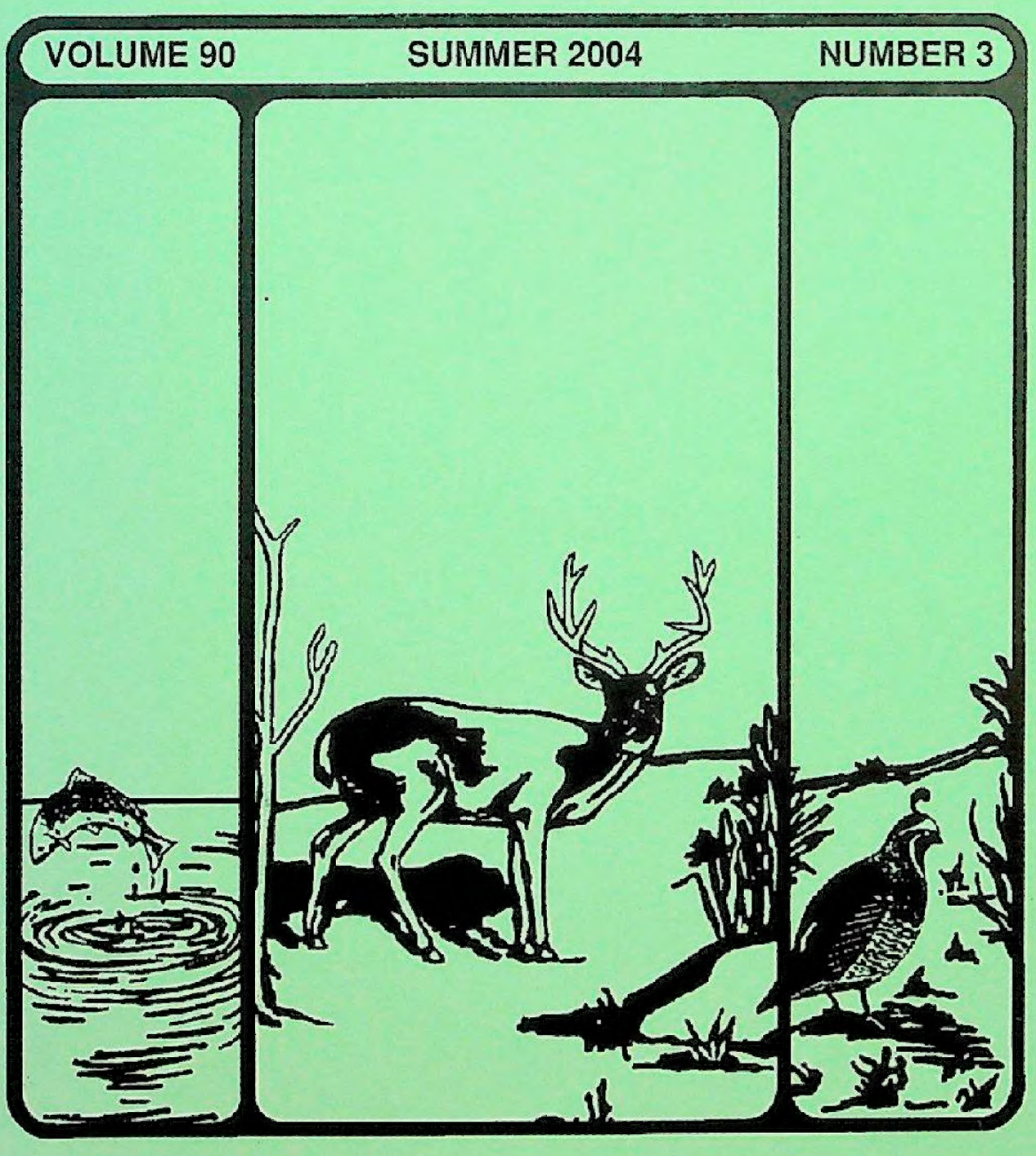
# CALIFORNIA FISH AND GAME

"CONSERVATION THROUGH EDUCATION"

VOLUME 90 SUMMER 2004 NUMBER 3



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Please direct correspondence to:

Ken Hashagen
Editor-in-Chief
*California Fish and Game*
P.O. Box 944209
Sacramento, California 94244-2090
e-mail: khashagen@mindspring.com

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VOLUME 90 SUMMER 2004 NUMBER 3

*Published Quarterly by*

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In Figure 3, the complete graph did not print.

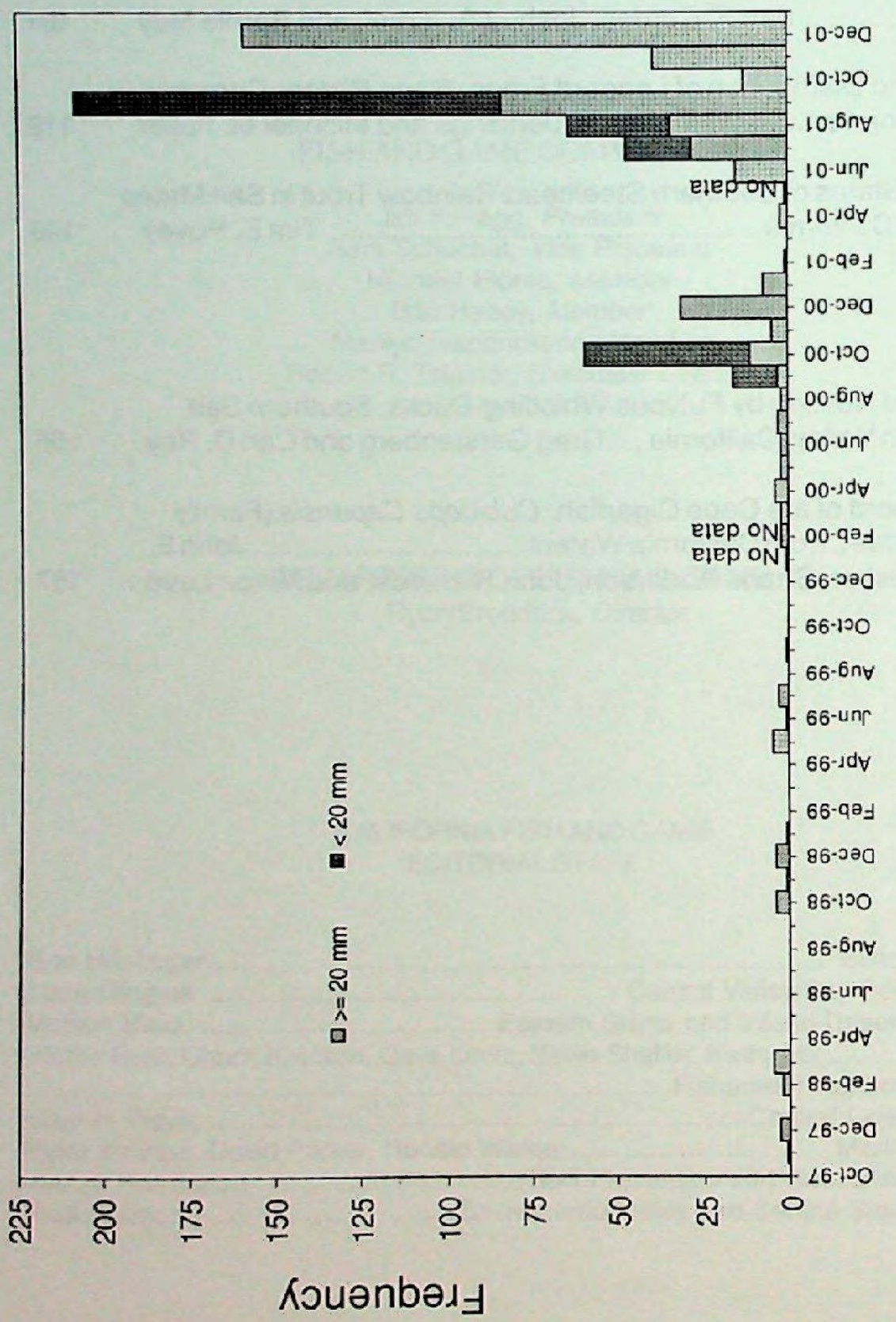


Figure 3. Shokihaze goby catch by month for two size classes (< 20 mm and = 20 mm TL) derived from otter trawl sampling by California Department of Fish and Game, San Francisco Bay Study (November 1997 through December 2001).

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# CONSERVATION OF PAIUTE CUTTHROAT TROUT: THE GENETIC LEGACY OF POPULATION TRANSPLANTS IN AN ENDEMIC CALIFORNIA SALMONID

JAN F. CORDES*, JOSHUA A. ISRAEL, and BERNIE MAY
Department of Animal Science
University of California, Davis
One Shields Avenue
Davis, CA 95616

**Paiute cutthroat trout, *Oncorhynchus clarki seleniris*, are threatened by loss of genetic diversity, restricted distributions, and historical hybridization with other introduced trout species. In this study, we used a single copy nuclear (scnDNA) marker and several nuclear microsatellites to assess levels of rainbow trout (RT) hybridization, estimate existing amounts of genetic variation, and characterize relationships among nine populations of Paiute cutthroat trout (PCT). No evidence of RT introgression was found in any of the PCT populations based on an scnDNA marker and five microsatellite loci. The two polymorphic microsatellite markers revealed population heterozygosities ranging from 0.138-0.657 (average=0.469) and 0.336-0.722 (average=0.544), respectively. Allele frequency distributions differed significantly from Hardy-Weinberg equilibrium in a single sample at one locus. Log-likelihood *G* tests and population pairwise $F_{ST}$ estimates indicated significant differentiation among most of the samples, and a neighbor-joing phenogram of $D_{CE}$ genetic distances revealed genetic relationships among populations that closely reflected PCT stocking history. Results support that efforts to eradicate hybridized PCT have been successful. However, the remaining populations are fragmented with limited genetic variation.**

## INTRODUCTION

The Paiute cutthroat trout, *Oncorhynchus clarki seleniris*, is a narrowly distributed form of cutthroat trout most closely related to the more widespread Lahontan cutthroat, *O. c. henshawi*, of eastern California, Nevada, and southern Oregon (Nielsen and Sage 2002). Historically, Paiute cutthroat trout (PCT) were probably restricted to an approximately 10-km stretch of Silver King Creek below Llewellyn Falls and above Silver King Canyon gorge (Fig. 1), which effectively isolated them from Lahontan cutthroat trout (LCT) inhabiting the adjacent East Fork Carson River. Over time, PCT evolved distinct phenotypic characteristics, including an almost complete lack of body spotting and an iridescent hue, which distinguished them from other cutthroat and rainbow trout (RT) populations. Snyder (1933, 1934) first recognized PCT as a subspecies based on

* Current address: Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, P.O. Box 1346, Gloucester Point, VA 23062, e-mail: jfcordes@vims.edu.

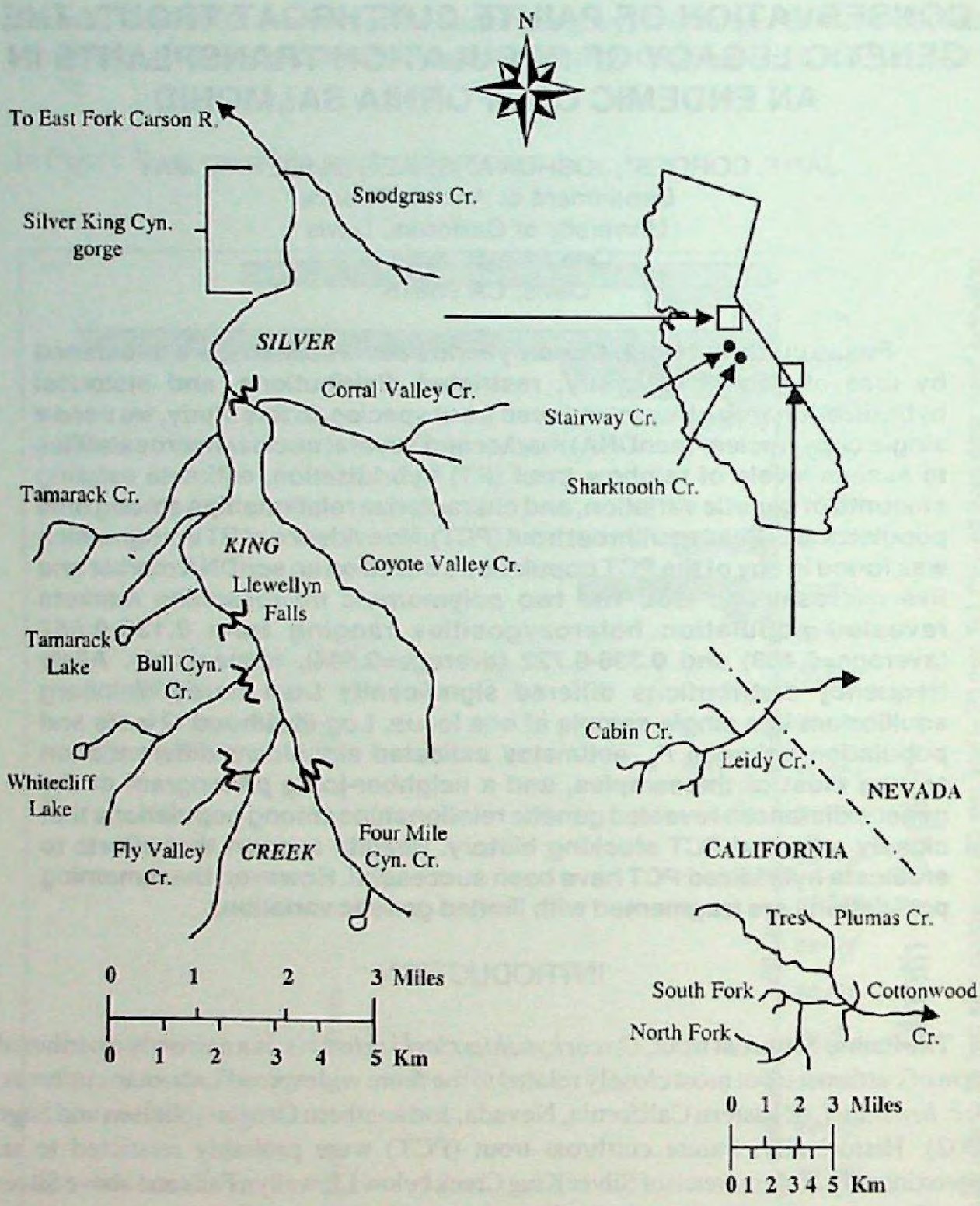


Figure 1. Map depicting the current distribution of Paiute cutthroat trout in California. Populations in Stairway Creek, Sharktooth Creek, and the White Mountains represent out-of-basin transfers from various locations in the Silver King Creek basin. Natural barriers to fish migration occurring in various streams are indicated with a wavy line.

the meristics of PCT populations that had been planted above Llewellyn Falls early in the last century.

The first documented transfer of PCT occurred in 1912, when a Basque sheepherder transplanted a small number of PCT from Silver King Creek below Llewellyn Falls (Fig. 2) into the fishless waters above (V. S. Connell; in Ryan and Nicola 19761). As early as 1924, putative hybrid LCT/PCT and RT/PCT were found in Silver King Creek below Llewellyn Falls (Ryan and Nicola 1976[1]). By the time PCT were described as a subspecies (Snyder 1933, 1934), it is probable that no natural, non-hybridized populations existed in its native historic range. Early in the last century, poorly documented, unauthorized transfers of PCT into fishless tributaries of Silver King Creek above various barriers (Fig. 1) included Four Mile Canyon Creek, Corral Valley Creek, and Coyote Valley Creek (Ryan and Nicola 1976[1]). These populations served as critical early refugia from introgression once unauthorized transfers of rainbow and LCT created hybrids in Silver King Creek below Llewellyn Falls. In 1947, the California Department of Fish and Game (CDFG) made additional intra-basin transfers from Silver King Creek above Llewellyn Falls into Fly Valley and Bull Canyon Creeks (Vestal 1964[2]). However, only the Fly Valley Creek transfer appeared successful (Ryan and Nicola 1976[1]). PCT have also been successfully transplanted out of their native range to other drainages in California (Fig. 1) including North Fork Cottonwood Creek and Cabin Creek in the White Mountains (Mono County), Stairway Creek (Madera County), and Sharktooth Creek (Fresno County). In 1949, an unauthorized introduction of RT resulted in hybridization of PCT above Llewellyn Falls. In addition, introductions of LCT into Silver King Creek may have occurred prior to 1949, and an accidental aerial planting into Whitecliff Lake is suspected to have occurred in 1955 (Ryan and Nicola 1976[1]). In 1964, PCT collected from Corral Valley and Coyote Valley Creeks were described as slightly hybridized with RT based on meristics (Ryan and Nicola 1976[1]).

The California Fish and Game Commission recognized the threats of hybridization and over-fishing to the endemic PCT soon after its discovery (Ryan and Nicola 1976[1]). The threat of extinction from hybridization and introgression with introduced rainbow and LCT resulted in the federal listing of PCT as endangered in 1970 (Federal Register 35:16047[3]), though it was downlisted to threatened in 1975 (Federal Register 40: 29863[4]) to allow for aggressive management. In 1974, after reviewing Snyder's original meristic description of *O. c. seleniris*, the CDFG realized that body spotting was an unreliable diagnostic tool for evaluating hybridization (Ryan and Nicola 1976[1]). The CDFG turned to genetic analysis of certain populations to detect hybridization and determine if

---

[1]Ryan, J.H. and S.J. Nicola. 1976. Status of the Paiute cutthroat trout, *Salmo clarki seleniris* Snyder, in California. California Department of Fish and Game Inland Fisheries Administrative Report #76-3. 56 pp.

[2]Vestal, E.H. 1964. Memo to Dick Beland. California Department of Fish and Game Memorandum. March 17, 1964. 1pp.

[3]Federal Register. 1970. Conservation of endangered species and other fish or wildlife. 35: 197(October 12,1970) p. 16047-16048.

[4]Federal Register. 1975. Threatened status for three species of trout. 40: 137 (July 16, 1975) p. 29863-29864.

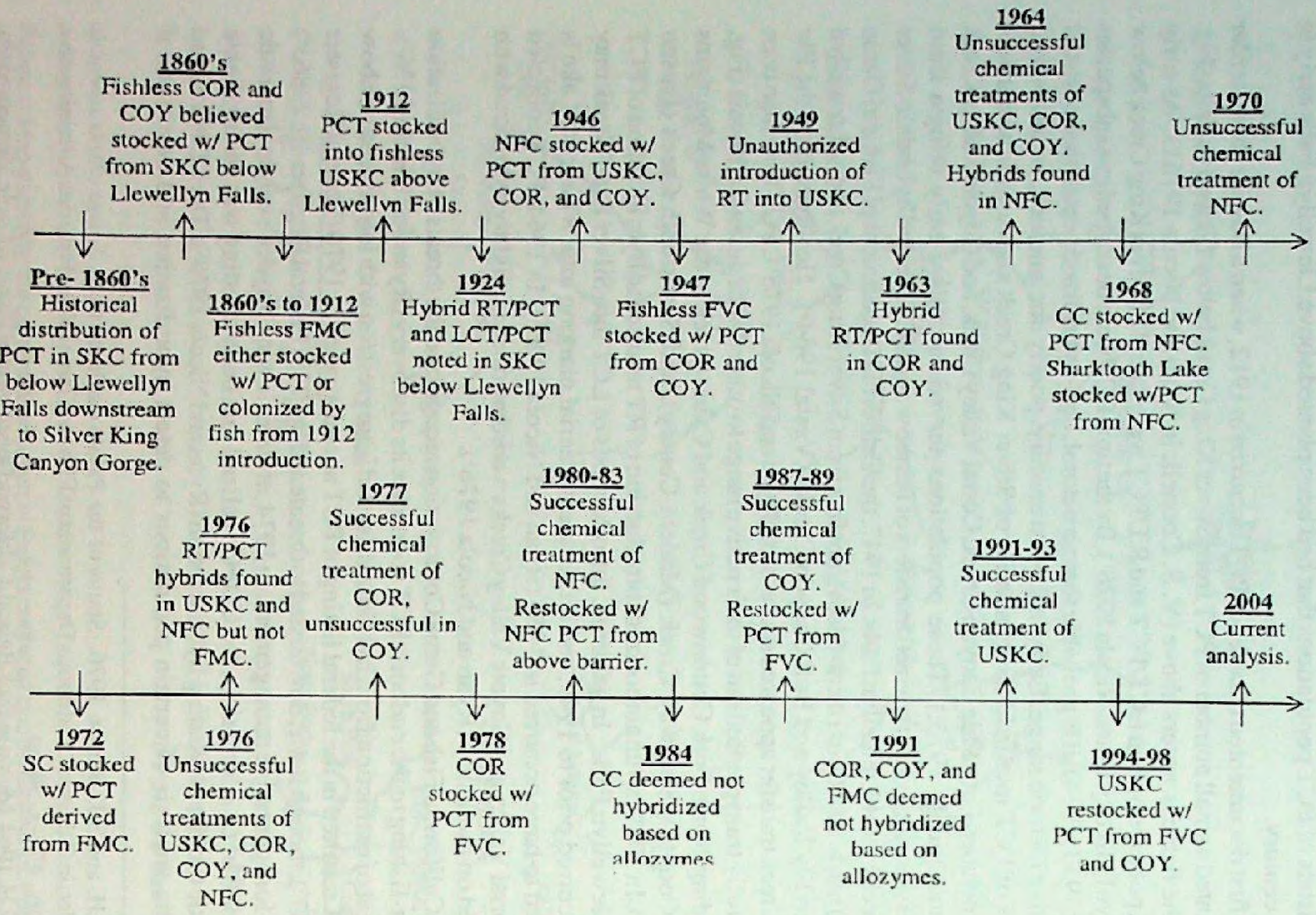
Pre- 1860's
Historical distribution of PCT in SKC from below Llewellyn Falls downstream to Silver King Canyon Gorge.
1860's
Fishless COR and COY believed stocked w/ PCT from SKC below Llewellyn Falls.
1860's to 1912
Fishless FMC either stocked w/ PCT or colonized by fish from 1912 introduction.
1912
PCT stocked into fishless USKC above Llewellyn Falls.
1924
Hybrid RT/PCT and LCT/PCT noted in SKC below Llewellyn Falls.
1946
NFC stocked w/ PCT from USKC, COR, and COY.
1947
Fishless FVC stocked w/ PCT from COR and COY.
1949
Unauthorized introduction of RT into USKC.
1963
Hybrid RT/PCT found in COR and COY.
1964
Unsuccessful chemical treatments of USKC, COR, and COY. Hybrids found in NFC.
1968
CC stocked w/ PCT from NFC. Sharktooth Lake stocked w/PCT from NFC.
1970
Unsuccessful chemical treatment of NFC.
1972
SC stocked w/ PCT derived from FMC.
1976
RT/PCT hybrids found in USKC and NFC but not FMC.
1976
Unsuccessful chemical treatments of USKC, COR, COY, and NFC.
1977
Successful chemical treatment of COR, unsuccessful in COY.
1978
COR stocked w/ PCT from FVC.
1980-83
Successful chemical treatment of NFC. Restocked w/ NFC PCT from above barrier.
1984
CC deemed not hybridized based on allozymes
1987-89
Successful chemical treatment of COY. Restocked w/ PCT from FVC.
1991
COR, COY, and FMC deemed not hybridized based on allozymes.
1991-93
Successful chemical treatment of USKC.
1994-98
USKC restocked w/ PCT from FVC and COY.
2004
Current analysis.

Figure 2. A timeline of important stocking and chemical treatment events involving Paiute cutthroat trout in the Silver King Creek basin and various out-of-basin transplant populations. Population codes follow those given in Table 1. Information taken from Vestal (1964), Ryan and Nicola (1976), Flint (1980), and Busack and Gall (1981) as cited in text.

various chemical treatments were successfully removing all introgressed fish. Allozyme analysis of PCT collected in 1976 found introgression by RT in upper Silver King Creek and North Fork Cottonwood Creek, but not in Four Mile Canyon Creek (Busack and Gall 1981). A 1976 chemical treatment of upper Silver King Creek was unsuccessful. In 1977, Corral Valley and Coyote Valley Creeks were treated, but only the Corral Valley treatment was deemed successful. Coyote Valley Creek was again treated during 1987-88 to remove hybrids. The North Fork of Cottonwood Creek was treated in 1980-81. Between 1991 and 1993, DFG chemically treated Silver King Creek in reaches where hybrids (based on body spotting and allozyme analysis) continued to be found. The repeated cycles of chemical treatment and restocking with presumably pure trout has been the main influence linking extant populations of PCT, so that current genetic relationships probably reflect stocking history rather than any pattern of natural affinities. Limitations of allozyme analyses (including limited variability and destructive sampling) and the continued uncertainties surrounding the genetic integrity of various PCT populations required non-invasive, highly polymorphic genetic tools to identify current threats of hybridization and introgression, and evaluate the success of restocking efforts.

The goals of this study were to use nuclear microsatellite and single copy nuclear (scn) DNA markers to investigate the genetic status of PCT in Silver King Creek, its tributaries, and out-of-basin populations. More specifically, the objectives were to: (1) evaluate potential markers to differentiate RT, LCT, and PCT, (2) survey PCT populations in the Silver King Creek drainage and out-of-basin transplants for evidence of RT introgression, and (3) characterize the genetic diversity within and among extant PCT populations.

## MATERIALS AND METHODS

### Sample Collection

Fin clip samples of PCT, LCT, and RT were collected in the summer months between 1996 and 2000 (Table 1). In 1996, California Department of Fish and Game (CDFG) personnel collected PCT samples from the North Fork of Cottonwood Creek. Also in 1996, U.S. Geological Survey/Biological Research Division (USGS/BRD) personnel collected LCT samples from the East Carson River, Murray Canyon Creek, and Poison Flat Creek. U.S. Forest Service (USFS) biologists collected samples of PCT from Sharktooth Creek in 1999 and Stairway Creek in 2000. All of the above samples were dried and stored in scale envelopes. Also in 2000, CDFG biologists collected fin clip samples from PCT at five locations in the Silver King Creek basin and Cabin Creek. They also collected two suspected hybridized PCT from upper Fish Valley (upper Silver King Creek above Llewellyn Falls) and one from Bull Canyon Creek (Table 1). Finally, CDFG personnel collected RT samples from the North Fork American River in 2000. Samples from the Silver King Creek basin, Cabin Creek, and the North Fork American River were placed in DMSO storage buffer (20% DMSO, 0.25 M EDTA, NaCl to saturation, pH 7.8), and stored at room temperature.

Table 1. Colection names, locations, sizes, and collection dates of Paiute cutthroat trout samples collected between 1996 and 2000. Location codes are given in parentheses.

| Species | Sample name | County | Sample size (n) | Collection date |
|---|---|---|---|---|
| Paiute cutthroat | Cabin Cr. (CC) | Mono | 26 | 08/23-24/2000 |
| | N. Fork Cottonwood Cr. (NFC) | Mono | 31 | 07/17/2000 |
| | Stairway Cr. (SC) | Madera | 30 | 08/04/2000 |
| | Sharktooth Cr. (STC) | Fresno | 25 | 08/22/2000 |
| | Corral Valley Cr. (COR) | Alpine | 33 | 08/11/2000 |
| | Four Mile Canyon Cr. (FMC) | Alpine | 31 | 08/14/2000 |
| | Coyote Valley Cr. (COY) | Alpine | 30 | 08/11/2000 |
| | Silver King Cr. (SKC) | Alpine | 28 | 08/12/2000 |
| | Fly Valley Cr. (FVC) | Alpine | 35 | 08/14/2000 |
| Suspected hybrids[a] | Upper Silver King Cr. (USKC) | Alpine | 2 | 08/27/2000 |
| | Bull Canyon Cr. (BCC) | Alpine | 1 | 08/27/2000 |
| Lahontan cutthroat | East Carson R. (ECR) | Alpine | 1 | 08/29/1996 |
| | Murray Canyon Cr. (MCC) | Alpine | 2 | 08/29/1996 |
| | Poison Flat Cr. (PFC) | Alpine | 3 | 08/30/1996 |
| Rainbow trout | N. Fork American R. (NFAR) | Placer | 6 | 08/03/2000 |

[a]Suspected Paiute cutthroat/rainbow trout hybrids based on body spotting (B. Somers, California Dept. of Fish and Game, personal communication)

## Genetic Analyses

Whole genomic DNA was extracted from fin clip samples using the Qiagen DNeasy™ Tissue Kit. To screen for polymorphisms, we assessed 11 microsatellite loci via the polymerase chain reaction (PCR) in six individuals each of PCT, LCT, and RT. Initially, 10 $\mu$L amplification reactions were carried out containing 4.85 $\mu$l sterile $dH_20$, 1.0 $\mu$l 10X PCR buffer, 0.3 $\mu$l 50 mM $MgCl_2$, 0.8 $\mu$l 2.5 mM dNTP mixture, 1 $\mu$l 10 mM forward primer, 1 $\mu$l 10mM reverse primer, 0.05 $\mu$l Taq I polymerase (0.25 U total), and 1 $\mu$l sample DNA (approximately 5 ng DNA total). PCR reactions were first denatured for 1 min 30 sec at 95°C, followed by steps of 1 min at 95°C, 30 sec at 52°C, and 1 min at 72°C for 30 cycles. PCR products were then electrophoresed on a 5% polyacrylamide denaturing gel for 1.5 hours at 35 watts, and alleles were visualized using a Molecular Dynamics 595 FluorImager. Allele sizes were estimated using the Molecular Dynamic's FragmeNT Analysis application (Version 1.1, 1993-4). Seven of the 11 microsatellite loci (Table 2) consistently amplified in PCT, LCT, and RT, and only 2 (OMM1058 and OMM1088) proved polymorphic in PCT.

PCR conditions for amplifying PCT DNA at the two polymorphic loci were optimized for use with fluorescent-labeled primers. The OMM1058 and OMM1088 forward primers were labeled with HEX and FAM fluorophores, respectively. PCR was carried out with 5.15 $\mu$l of sterile $H_20$, 1.0 $\mu$l 10X PCR buffer, 0.8 $\mu$l 2.5 mM dNTP mixture, 0.4 $\mu$l

Table 2. Characteristics of 7 microsatellite loci evaluated for use in this study. Size ranges are given in base pairs (bp). All microsatellite primer sets are from Rexroad et al. (2001).

| Microsatellite | Paiute Cutthroat Trout | | Lahontan Cutthroat Trout | | Rainbow Trout | |
|---|---|---|---|---|---|---|
| Loci | # alleles | Size range | # alleles | Size range | # alleles | Size range |
| OMM 1050 | 1 | 220 | 4 | 224-236 | 8 | 240-280 |
| OMM 1051 | 1 | 428 | 5 | 400-496 | 7 | 232-276 |
| OMM 1058 | 4 | 216-228 | 3 | 208-216 | 7 | 184-240 |
| OMM 1086 | 1 | 194 | 3 | 194-202 | 5 | 194-242 |
| OMM 1088 | 6 | 204-224 | 5 | 144-240 | 5 | 120-140 |
| OMM 1104 | 1 | 224 | 1 | 240 | 5 | 168-216 |
| OMM 1108 | 1 | 156 | 6 | 144-176 | 4 | 148-188 |

50 mM $MgCl_2$, 0.05 $\mu$l Taq I polymerase (0.25 U total), 0.2 $\mu$l of 1 mM labeled forward primer, 0.4 $\mu$ of 10 mM reverse primer, and 2 $\mu$l PCT sample DNA (approximately 10 ng total). PCR amplification conditions were the same as described above. Alleles were separated electrophoretically on a 5.5% polyacrylamide gel using the MJ Research BaseStation gel analysis system (MJ Research, Inc., Boston, Ma, USA) and analyzed using MJ Research's Cartographer software. The Genescan 500 size standard (MJ Research) labeled with ROX fluorescent dye was run in each lane.

An anonymous single copy nuclear (ascnDNA) marker was developed following protocols established in the Genomic Variation Laboratory at the University of California, Davis (Tranah et al. 2003). Briefly, 200 ng of DNA from RT, LCT, and PCT samples were digested for 1 hour at 37 °C with the restriction enzymes EcoRI and Mse I using 0.1 $\mu$l of each enzyme, 2.0 $\mu$l 10X buffer, 0.16 $\mu$l 400X BSA, and $ddH_2O$ to 16 $\mu$l. Adapters to Mse I and EcoRI restriction sites were then ligated to the ends of the DNA fragments by adding 0.4 $\mu$l Eco Adapter (5 pmoles), 0.4 $\mu$l Mse Adapter (50 pmoles), 0.2 $\mu$l 100mM ATP, 0.2 $\mu$l T4 DNA Ligase, 0.4 $\mu$l 10x buffer, 0.1 $\mu$l 400X BSA, and 2.3 ml $H_2O$ to the 16 ml reaction. The ligation reaction was performed overnight at room temperature, after which 180 $\mu$l TLE was added. The adapters were designed to have a final 3' nucleotide that was different from the original sequence, thus destroying the original restriction site. The fragments were then amplified via PCR using primers complimentary to the adapter sequences. The primers used were designed with one to three varying nucleotides overhanging the 3' end of the adapters. Fragments were selectively amplified by varying the specific primers that were used. Fragments were first amplified using shorter, less specific preamp primers. The PCR conditions were: 94°C for 1.5 minutes, followed by 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute for 23 cycles; 180 $\mu$l of TLE were added to the preamp PCR product for each sample. Fragments were then amplified with different combinations of the specific primers, using a touchdown PCR program with a denaturing step of 94°C for 30 seconds, an annealing step beginning at 65°C for the first cycle and decreasing 0.7°C each cycle until 56°C for 30 seconds, and an elongation step of 72°C for 1 minute. Thirty-five cycles

were performed for the entire reaction. After amplification, the products were electrophoresed on a 5% acrylamide denaturing gel for 1.5 hours at 35 W, and scanned with a Molecular Dynamics 595 FluorImager. Bands of PCR-amplified product that appeared fixed and unique to a single subgroup (RT, LCT, or PCT) were cut out of the denaturing gels, and re-suspended in 200 $\mu$l TLE. These fragments were re-amplified using non-labeled primers under preamp PCR conditions and sequenced using Perkin Elmer/ABI BigDye Dye Terminator chemistry. New primers were designed that specifically amplified the potentially informative fragments using the software Primer Select (DNAStar, Inc.).

The sequences generated from these fragments were used to design new primers. From the sequenced alleles described above, we developed primers for the single locus AGGCCG-3 (F: 5'-GGGGCAAAGGGGGTCTGAC-3'; R: 5'-AACCGGGGGTTGACGTACATTATA-3'), which exhibited a definitive allele size difference between RT and LCT/PCT. No fragments exhibiting size differences between LCT and PCT were found. The AGGCCG-3 locus was used to investigate the possibility of RT introgression in the PCT populations sampled in this study. The PCR conditions for amplification of this locus were: 94°C for 2 minutes, followed by steps of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds for 30 cycles, and a final extension step of 72°C for 5 minutes after cycling. PCR products were then electrophoresed on a 5% polyacrylamide denaturing gel for 1.5 hours at 35 W, and imaged with a Molecular Dynamics 595 FluorImager. Allele sizes were estimated using Molecular Dynamic's FragmeNT Analysis application.

## Statistical Analyses

Standard molecular indices including allele frequencies, expected heterozygosities ($H_e$), and observed heterozygosities ($H_o$) were calculated using the program Genes in Populations[5]. Conformance to Hardy-Weinberg expectations for each locus/population combination was tested by the Markov chain method to obtain unbiased estimates of Fisher's exact test based on 1000 iterations (Rousset and Raymond 1995) using the web-based software program Genepop on the Web (available at http://wbiomed.curtin.edu.au/genepop/genepop_op1.html). Log-likelihood $G$ tests using the Markov chain method for population differentiation at each locus and for all loci combined, as well as calculations of Weir and Cockerham's (1984) estimates of $F_{ST}$ were also performed using Genepop on the Web. Statistical significance of population pairwise $F_{ST}$ values was determined using the software program FSTAT 2.9.3 (updated from Goudet 1995; available at http://www.unil.ch/izea/softwares/fstat.html). All significance values resulting from multiple comparisons were corrected for Type I error using the Bonferroni correction of Rice (1989)

[5]Genes in Populations 2.2. Designed by B. May and C. Krueger and written in C by W. Eng and E. Paul; program available for download at http://animalscience.ucdavis.edu/extension/Gene.htm

Cavalli-Sforza and Edward's (1967) chord distances ($D_{CE}$) were calculated among samples and plotted as a neighbor-joining (NJ) phenogram (Saitou and Nei 1987) using Phylip 3.5c (Felsenstein 1995). The original allele frequency matrix was then resampled 1000 times by bootstrapping and the genetic distances between samples were estimated for each resulting matrix. A consensus UPGMA diagram was generated and all bootstrap values above 50% were plotted on to the dendrogram from the original sample matrix to indicate stability of the nodes.

## RESULTS

### Hybridization Assessment

Sixty-four AFLP primer combinations were used to screen for genetic differences among PCT, LCT, and RT. From these 64 combinations, 87 potentially informative fragments were cut out and sequenced. From these sequences, primers to amplify 23 novel loci were developed. Only one (AGGCCG-3) of the 23 novel loci screened showed fixed differences between PCT and RT, while none showed fixed differences between Paiute and LCT. All RT screened for the AGGCCG-3 locus shared a single band 219 base pairs (bp) in length, while all PCT and LCT samples exhibited a single band of 189 bp. All individuals from each PCT collection (Table 1) were amplified at the AGGCCG-3 locus and no rainbow-type bands were found, indicating that none of the tested samples were a product of hybridization with RT.

Of the seven microsatellite loci screened (Table 2), one (1088) was diagnostic, exhibiting disjunct distributions of alleles between PCT and RT. As in the AGGCCG-3 marker above, no rainbow-type bands were found in any of the PCT collections tested. This is consistent with the findings from the five microsatellite loci (OMM1050, OMM1051, OMM 1086, OMM1104, and OMM1108) that were monomorphic in PCT. Five of these loci were polymorphic in RT (Table 2), and none of the RT alleles at any locus were found in 75 tested individuals composed of subsets of eight samples from each PCT population and the suspected hybrids.

Four of the microsatellite loci (Table 2) monomorphic in PCT were polymorphic in LCT and some may be useful as diagnostic markers for identifying instances of hybridization between the two subspecies. However, since the present study focused on identifying diagnostic markers between PCT and RT, we limited our survey of LCT to fish derived from the East Carson River. A more extensive survey of LCT populations in California and Nevada would be necessary to assess the usefulness of the AGGCCG-3 or microsatellite markers in distinguishing PCT From LCT. We chose the North Fork American River population as our reference RT samples because it is a wild population shown to contain nearly all of the diversity found in three different strains of hatchery RT, including the Hot Creek strain that was the source of RT introduction into Silver King Creek above Llewellyn Falls in 1949 (J.F. Cordes, unpublished data based on a survey of six microsatellite loci.).

## Genetic Variation and Distances

For the seven microsatellite loci listed in Table 2, all but two were monomorphic in PCT. The number of alleles per locus for polymorphic OMM1058 and OMM1088 was 4 and 6, respectively. Since a goal of the microsatellite analysis was to evaluate variability between PCT populations, we did not use the numerous monomorphic loci for the rest of the analyses. Microsatellite variation was therefore evaluated at two loci, OMM1058 and OMM1088.

Allele frequencies, observed heterozygosity ($H_o$), and expected heterozygosity ($H_e$) are listed in Table 3. Allele sizes for the OMM1058 locus ranged from 216-228 bp. All four of the OMM1058 alleles were found in the North Fork Cottonwood Creek sample; all other populations had three alleles present. Observed heterozygosities ($H_o$) for OMM1058 ranged from 0.138 (Stairway Creek) to 0.657 (Fly Valley Creek), with an average of 0.469. Allele sizes for the OMM1088 locus ranged from 205-225 bp. Although all of the populations contained a majority of the six OMM1088 alleles, none contained all of them (Table 3). The observed heterozygosities ($H_o$) at this locus ranged from 0.336 (Stairway Creek) to 0.722 (Cabin Creek), with an average of 0.544. The only significant deviation ($P<0.05$) from Hardy-Weinberg expectations after correction for multiple tests occurred at the OMM1088 locus in the Sharktooth Creek collection.

Table 3. Allele frequencies, sample sizes (n), observed ($H_o$) and expected ($H_e$) heterozygosities, and $P$ values for tests of Hardy-Weinberg equilibrium for nine populations of Paiute cutthroat trout. Location codes are given in Table 1. An (*) indicates a significant deviation from Hardy-Weinberg equilibrium ($P<0.01$) after correction for multiple tests (initial a= 0.001).

| Locus | Allele | CC | NFC | SC | STC | COY | COR | FMC | SKC | FVC |
|---|---|---|---|---|---|---|---|---|---|---|
| **OMM1058** | 216 | - | 0.067 | - | - | - | - | - | - | - |
| | 220 | 0.389 | 0.117 | - | 0.200 | 0.121 | 0.387 | 0.050 | 0.143 | 0.186 |
| | 224 | 0.361 | 0.800 | 0.931 | 0.680 | 0.586 | 0.323 | 0.800 | 0.571 | 0.414 |
| | 228 | 0.250 | 0.017 | 0.069 | 0.120 | 0.293 | 0.290 | 0.150 | 0.286 | 0.400 |
| | n | 18 | 30 | 29 | 25 | 31 | 30 | 29 | 28 | 35 |
| | $H_o$ | 0.611 | 0.333 | 0.138 | 0.480 | 0.643 | 0.581 | 0.333 | 0.657 | 0.657 |
| | $H_e$ | 0.656 | 0.342 | 0.128 | 0.483 | 0.571 | 0.662 | 0.335 | 0.634 | 0.634 |
| | P | 0.098 | 0.174 | 1.000 | 0.365 | 0.138 | 0.587 | 1.000 | 0.680 | 0.966 |
| **OMM1088** | 209 | 0.417 | 0.536 | - | 0.405 | - | - | - | - | - |
| | 213 | 0.083 | 0.018 | 0.150 | 0.095 | 0.033 | - | 0.100 | 0.259 | 0.156 |
| | 217 | 0.306 | 0.411 | 0.633 | 0.357 | 0.650 | 0.470 | 0.400 | 0.667 | 0.641 |
| | 221 | 0.111 | 0.036 | - | 0.024 | 0.167 | 0.061 | - | 0.074 | 0.125 |
| | 225 | 0.083 | - | 0.217 | 0.119 | 0.150 | 0.470 | 0.483 | - | 0.078 |
| | 229 | - | - | - | - | - | - | 0.017 | - | - |
| | n | 18 | 28 | 30 | 21 | 33 | 30 | 30 | 27 | 32 |
| | $H_o$ | 0.722 | 0.417 | 0.336 | 0.383 | 0.525 | 0.609 | 0.483 | 0.61 | 0.563 |
| | $H_e$ | 0.681 | 0.442 | 0.329 | 0.584 | 0.527 | 0.608 | 0.466 | 0.589 | 0.543 |
| | $P$ | 0.075 | 0.444 | 0.142 | **0.000*** | 0.434 | 0.224 | 1.000 | 0.537 | 0.800 |

Log-likelihood *G* tests revealed significant genetic differentiation among populations over all for both loci individually and combined. Population pairwise $F_{st}$ values indicated significant differentiation among all samples with the following exceptions: Coyote Creek and Fly Valley Creek; Silver King Creek and Fly Valley Creek; Stairway Creek and Four Mile Canyon Creek; Sharktooth Creek and Cabin Creek; and Sharktooth Creek and North Fork Cottonwood Creek (Table 4).

Table 4. Population pairwise estimates (above diagonal) and log likelihood *G* tests of allele frequency differences (below diagonal) for two microsatellite loci. An (*) above the diagonal indicates a statistically significant population pairwise $F_{ST}$ value; numbers below the diagonal indicate loci (1=OMM1058, 2=OMM1088) exhibiting a significant difference in allele frequencies. Results based on $P<0.05$ after correction for multiple tests (initial a=0013).

| Population | COR | COY | SKC | FVC | FMC | SC | STC | CC | NFC |
|---|---|---|---|---|---|---|---|---|---|
| COR | - | **0.089*** | **0.146*** | **0.076*** | **0.144*** | **0.246*** | **0.137*** | **0.093*** | **0.271*** |
| COY | 2 | - | **0.029*** | 0.008 | **0.114*** | **0.093*** | **0.090*** | **0.138*** | **0.175*** |
| SKC2 | 2 | - | 0.017 | **0.149*** | **0.121*** | **0.102*** | **0.153** | **0.193*** | |
| FVC2 | 0 | 0 | - | **0.150*** | **0.165*** | **0.108*** | **0.109*** | **0.212*** | |
| FMC | 1,2 | 2 | 2 | 1,2 | - | 0.076 | **0.104*** | **0.213*** | **0.208*** |
| SC 1,2 | 1 | 1,2 | 1 | 0 | - | **0.137*** | **0.297*** | **0.186*** | |
| STC1,2 | 2 | 2 | 2 | 2 | 1,2 | - | 0.040 | 0.016 | |
| CC 2 | 2 | 2 | 2 | 1,2 | 1,2 | 0 | - | **0.142*** | |
| NFC1,2 | 1,2 | 1,2 | 1,2 | 1,2 | 1,2 | 0 | 1 | - | |

In order to examine the relatedness between populations, a NJ phenogram was constructed based on $D_{CE}$ genetic distances. This phenogram and a graphical depiction of stock transfers are detailed in Figure 3. All of the nodes were supported with bootstrap values greater than 50% (range 51-76%), and genetic relationships among populations closely followed their stocking histories. This concordance in the data between genetic relationships and stocking history lent confidence to our results despite the relatively few number of microsatellite markers employed; however, a more extensive analysis of additional samples using a larger suite of markers would help to verify our initial findings.

## DISCUSSION

### Hybridization Assessment

The AGGCCG-3 locus was developed to distinguish RT from PCT. The fixed allele for PCT was the only allele observed in all samples from every PCT population as well as the suspected hybrids (Table 1) from Bull Canyon Creek and Silver King Creek. No rainbow-type alleles were found in any of the PCT samples. Although it has been

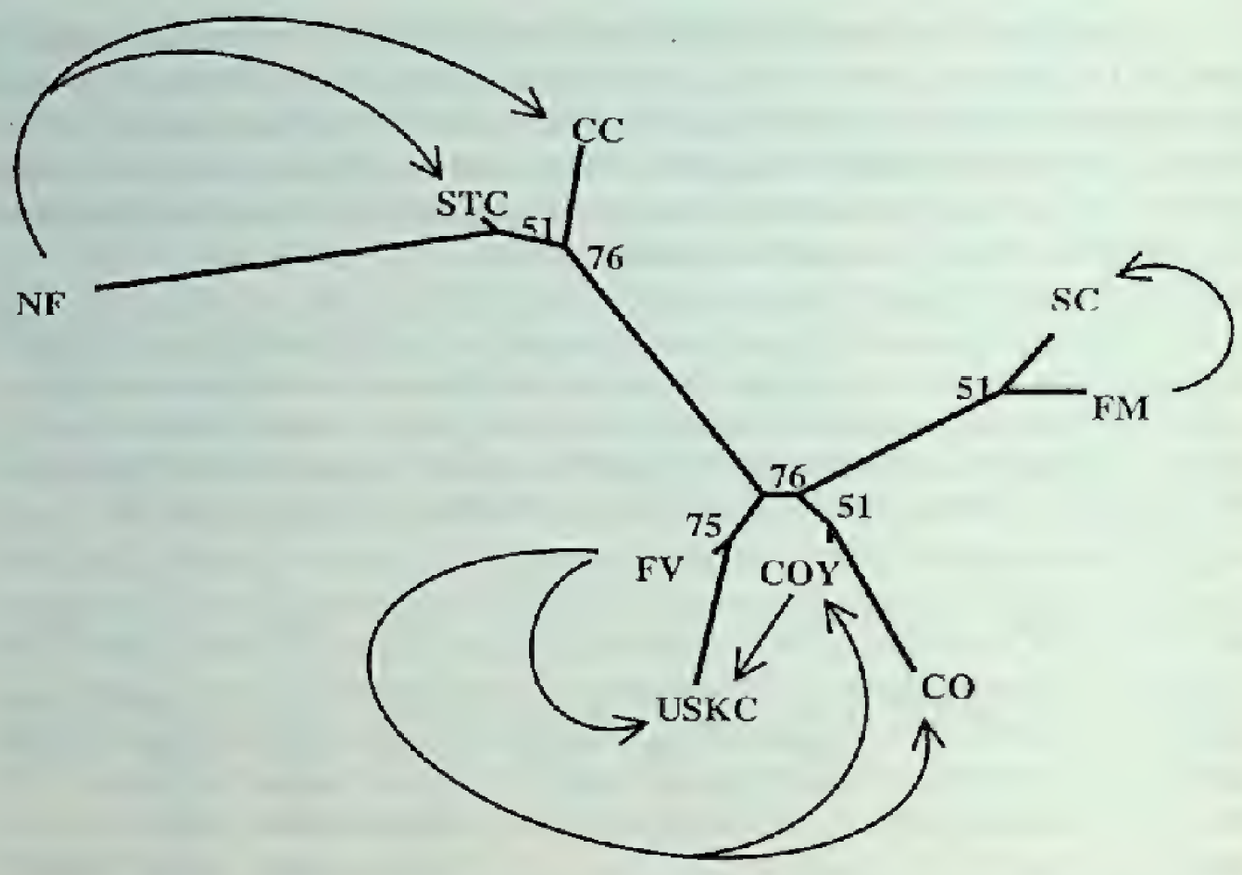


Figure 3. Neighbor-joinung tree depicting relationships among nine creek populations of Paiute cutthroat trout based on Cavalli-Sforza and Edwards $D_{CE}$ genetic distances. Bootstrap values at the nodes indicate the percentage of times populations beyond the node grouped together based on 1,000 iterations. Arrows correspond to direction of most recent stocking from source to current populations sampled in this study (discussed in text).

suggested that the Cabin Creek population may be introgressed (USFWS 1985[6], p.13), no rainbow-type band was found in any individuals from Cabin Creek. These results suggest that none of the tested PCT populations have undergone recent hybridization with RT. Introgression from past hybridization events may be difficult to detect when relying on a single genetic marker, but such introgression seems unlikely since the absence of RT alleles in any of the PCT populations for the polymorphic 1088 microsatellite locus or the five monomorphic loci supports the conclusion that hybridization has not occurred. Thus it appears that the combination of chemical and electro-shocking treatments that have taken place since hybridization was last genetically assessed (Busack and Gall 1981) has been successful at eradicating PCT/RT hybrids to below detectable levels from all of the tested populations in Silver King Creek basin and the out-of-basin transfers.

## Genetic Variation and Stocking History

This study detected limited genetic variability in PCT. Of seven microsatellite loci screened, only two were polymorphic in PCT (average number of alleles/locus=2.14),

in contrast to the six loci found to be polymorphic in a small sample of LCT from the neighboring East Fork Carson River (average number alleles/locus-= 3.86). Higher levels of microsatellite variability in larger sample sizes of LCT have been reported by Nielsen and Sage (2002) who screened 16 amplifiable loci derived from various trout and salmon species and found 13 to be polymorphic (average number of alleles/locus of 15.0). Higher levels of microsatellite variability based on average number of alleles/locus and observed heterozygosities have also been shown in other native California trout including California golden trout (Cordes et al. 2001[7]) and redband trout (Nielsen et al. 1999), arguably making PCT the most narrowly distributed and genetically limited native trout in the state. This limited genetic variability may be due to a bottleneck associated with the original isolation of PCT from a common ancestor with the LCT, bottlenecks resulting from the small number of fish typically used as transplant stocks, or a combination of the two.

Log-likelihood *G* tests and population pairwise $F_{st}$ values revealed significant genetic differentiation among a majority of the samples. Given the stocking history of the basin, this probably represents the result of founder effects and genetic drift on populations started with small numbers of stocked fish, rather than naturally occurring differentiation of reproductively isolated native populations. All non-significant estimates of population differentiation (Coyote Creek and Fly Valley Creek; Silver King Creek and Fly Valley Creek; Stairway Creek and Four Mile Canyon Creek; Sharktooth Creek and Cabin Creek; and Sharktooth Creek and North Fork Cottonwood Creek) occurred between samples that shared common recent stocking histories (see discussion on stocking groups below).

Genetic distances suggest that three relatively discrete stocking groups of PCT currently exist, and that relationships among populations have been largely determined by stocking history (Fig. 3). The first group included fish from North Fork Cottonwood, Cabin, and Sharktooth Creeks. A second group was comprised of samples from upper Silver King, Fly Valley, Corral Valley, and Coyote Creeks. The third group consisted of samples from Stairway and Four Mile Canyon Creeks.

The first group of samples from North Fork Cottonwood Creek, Sharktooth Creek, and Cabin Creek were relatively distinct from all remaining samples on the NJ tree. The North Fork Cottonwood Creek samples may represent the oldest extant PCT population (but see discussion on Four Mile Canyon Creek fish). Established in 1946, this was the first out-of-basin transfer of PCT from upper Silver King, Coyote Valley, and Corral Valley Creeks (Vestal 1947). This transfer occurred prior to the introduction of RT above Llewellyn Falls from Hot Creek Hatchery in 1949; the fish collected from Corral Valley and Coyote Valley Creeks are believed derived from PCT planted by loggers in the late 1860's (Vestal 1964[2]). All three of these donor populations have since been extirpated by hybridization and subsequent chemical treatment.

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[6]U.S. Fish and Wildlife Service. 1985. Paiute Cutthroat Trout Recovery Plan. U.S. Fish and Wildlife Service, Portland, OR. 68 pp.

[7]Cordes, J.F., M.A. Blumberg, G.A.E. Gall, and B. May. 2001. Genetic status of California golden trout populations in the headwaters of Golden Trout Creek. Report to the California Department of Fish and Game Threatened Trout Committee. September 2001. 34 pp.

The North Fork Cottonwood Creek population was the only sample to have all four alleles at the OMM1058 locus, suggesting that the slightly higher level of variation within this sample may reflect its history as the oldest known remaining population. At the OMM1088 locus, we observed four out of six alleles. Interestingly, the 225 base pair allele at the OMM1088 locus was not found in this population, but was found in Cabin and Sharktooth Creeks, two out-of-basin transfers made with PCT from North Fork Cottonwood Creek. Each of these translocated populations had five alleles at this locus. Comparison of allele frequencies at this locus between the North Fork Cottonwood Creek sample and both of these populations reveals that the same two alleles (209 and 217 bp) account for more than 70% of the variation in all three samples. One of these alleles (209 bp) is not found in any of the other sample populations, and may be a relict of translocation from the now-extirpated upper Fish Valley populations. The Sharktooth Creek population was established in 1968 from 29 fish (23 from N. Fork Cottonwood Creek and six from Delaney Creek) placed into a headwater lake (Sharktooth Lake; Ryan and Nicola 1976[1]). By 1975, a survey of the lake found no fish, though PCT had apparently become established in the creek[8].

Apparently, an unauthorized transfer of RT led to putative hybrids becoming prevalent in North Fork Cottonwood Creek as far as a natural barrier in the Granite Meadow area by 1964. In 1970, the creek was electrofished and chemically treated; 75% of the fish (those with fewer than five spots) were returned to the stream and a survey in 1973 found no evidence of heavily spotted fish (putative hybrids). However, Busack and Gall (1981) described genetic evidence of introgression between PCT and RT in a 1976 collection taken from this stream below the Granite Meadow barrier. North Fork Cottonwood Creek was chemically treated below Granite Meadow in 1980-81, and fish were relocated downstream from above the barrier in 1982-83. In the present study, no evidence of hybridization was observed in the North Fork Cottonwood Creek sample taken from above the Granite Meadow barrier.

The second group on the NJ tree consisted of four loosely associated samples from upper Silver King, Fly Valley, Coyote, and Corral Valley Creeks. As mentioned previously, Silver King Creek above Llewellyn Falls was first stocked with presumably pure PCT in 1912. PCT in Fly Valley Creek were established in 1947 (Vestal 1964[1]) from a transfer of 54 fish; Ryan and Nicola (1976: p. 30) "assumed that it [the source] was either Coyote or Corral Valley Creeks or both." It is believed that Coyote and Corral Valley Creeks were originally fishless, but stocked by loggers in the late 1860's (Vestal 1964[1]), also with presumably pure PCT from below Llewellyn Falls. Upper Silver King Creek above Llewellyn Falls received its first transfer of RT in 1949, and a selective sportfishery and repeated electrofishing were ineffective at removing rainbow and hybrid trout after 13 years (Flint et al. 1998). Single chemical treatments were not

[8]It is unclear why the only significant departure from Hardy-Weinberg equilibrium occurred at the OMM1088 locus in this sample ($H_o$=0.383, $H_e$=0.584, $P$<0.001). The presence of a null allele (one that doesn't amplify via PCR due to sequence changes in the flanking regions where the primers bind) is possible, although it seems unlikely that the allele would be rare or absent in all other populations.

successful at removing putative hybrids in 1964 and 1976, and a chemical treatment project took place between 1990-1993 to remove any remaining hybrids in this reach of stream. In 1994-1998, pure PCT were relocated back in to upper Silver King Creek from Coyote Valley and Fly Valley Creeks. The location of the upper Silver King population in this group reflects this shared ancestry. However, these translocations were not genetically complete, since the OMM1088/225 bp allele present in both the Coyote Valley Creek and Fly Valley Creek samples is missing in the upper Silver King Creek sample. Also, allele frequencies differed between these three populations, although they continued to share the most common allele at both the OMM1058 and OMM1088 loci.

The Coyote Valley Creek population was re-established from the Fly Valley Creek population after successive chemical treatments in 1987 and again in 1988. This followed unsuccessful treatments in 1964 and 1977. Fifty-four PCT were transferred from Fly Valley Creek into Coyote Valley Creek in 1989. The decreased observed heterozygosity seen in the Coyote Valley Creek population suggests inbreeding as a result of the small initial number of transferred fish, although there was no loss of alleles at either loci when compared to the Fly Valley source population. Although the majority of the fish (87%) used to establish the upper Silver King Creek population came from Coyote Valley Creek, the upper Silver King Creek sample aligned more closely with the Fly Valley Creek sample in the dendrogram, suggesting differential reproduction, random drift, or both have affected the relationships among these three populations.

The most recent chemical treatment of Corral Valley Creek was in 1977 with a subsequent restocking of twenty fish from Fly Valley Creek. An initial decrease in population is believed to have occurred, as few fish were observed the year following the initial transfer (Bacon 1978[9]). Since the 1977 treatment, possible putative hybrids were identified and removed. In a comparison of allele frequencies between the samples from Fly Valley and Corral Valley Creeks, we observed the loss of the OMM1058 213 bp allele in the Corral Valley Creek sample. This and the fixation of the 225 bp allele at the OMM1088 locus in the Corral Valley Creek sample suggests a bottleneck likely occurred when this population was re-established.

The third cluster consists of Stairway and Four Mile Canyon creeks. The out-of-basin Stairway Creek population was established in 1972 from 77 Delaney Creek fish. The source populations for Delaney Creek were Four Mile Canyon Creek (n=43) and Fly Valley Creek (n=3). This translocation explains the grouping of Stairway Creek with Four Mile Canyon Creek. Both of these populations have four alleles at the OMM1088 locus, although the Four Mile Canyon Creek population contains an allele not represented in any other population. The Stairway Creek population has lost a single allele at the OMM1058 locus relative to its source populations, and only has two alleles. This population has the lowest overall heterozygosity of any population at both the OMM1088 ($H_o$=0.336) and OMM1058 ($H_o$=0.138) loci.

[9]Bacon, M. 1978. Coyote Valley and Corral Valley Creeks, Alpine County, California Department of Fish and Game Memorandum. October 20, 1978. 1 pp.

PCT in Four Mile Canyon Creek were either established by stockmen prior to 1912 (letter from C.S. Kabel to Elden Vestal, September 18, 1956; in Ryan and Nicola 1976) or ascended into this reach after the 1912 introduction into upper Silver King Creek above Llewellyn Falls. If these fish naturally colonized Four Mile Canyon Creek from upper Silver King Creek (before beaver dams presented a series of barriers to migration) they may represent the oldest continuous population of PCT. Electrofishing and removal of spotted trout was done between 1972 and 1992 and allozyme analysis in 1976 and 1991 did not produce evidence of RT introgression. About 1 mile of upstream habitat known to contain fish was never electrofished (William Somer, CDFG, personal communication).

PCT probably diverged from a more widely distributed PCT/LCT ancestor after a presumably small subpopulation of fish was isolated from the rest of the East Fork Carson River drainage by natural barriers in a gorge in lower Silver King Creek Canyon. Although the limited genetic diversity in PCT revealed in this study may have resulted from this initial separation, it has almost certainly been exacerbated by the repeated chemical treatment and restocking of virtually all of the extant populations. Fragmentation of an already narrowly distributed subspecies such as PCT into smaller, isolated populations can lead to accelerated genetic drift, resulting in random shifts in allele frequencies among populations, loss of genetic diversity, fixation of different alleles in different populations, and genetic diversification (fragmentation) of replicate populations originally from a single source (Frankham et al. 2002). Spruell et al. (1999) argued that local populations of Bull trout faced a high risk of extinction based on a significant degree of genetic differentiation and low numbers of spawners. Results of this study show that loss of genetic diversity, shifts in allele frequency, and genetic differentiation (fragmentation) is already apparent in PCT populations. In light of these findings, extant populations of PCT should be considered part of a single management unit with regard to planned restoration. The translocation of hundreds of fish from Coyote Valley and Fly Valley Creeks into upper Silver King Creek, and the apparent loss of an allele in the upper Silver King sample, displays the difficulty of estimating what contribution is necessary from donor populations when restocking fishless habitat. Future translocation efforts should ideally consist of large numbers of fish from multiple donor populations containing as much genetic variation as possible in order to minimize loss of diversity and the effects of inbreeding. Ultimately, management goals should include reconnection of as many of the extant populations in the Silver King Creek basin as possible. Efforts to reconnect PCT populations above Llewellyn Falls and re-establish uncompromised populations from below the falls downstream to the gorge in Silver King Creek Canyon are already in the planning stage (Will Somer, CDFG, personal communication). Out-of-basin PCT populations such as North Fork Cottonwood Creek and Four Mile Canyon Creek may constitute an important part of any restoration efforts, since unique alleles were found in both of these samples. Additionally, the development of molecular markers that can distinguish between LCT and PCT would be important for determining their genetic relationship and investigating the possibility of introgressive hybridization between the two groups prior to any restorations.

## ACKNOWLEDGMENTS

We would like to thank B. Somer, D. Wong, and D. Becker from the CDFG, and P. Strand from the USFS for providing the samples of PCT used in this study. K. Sage from the USGS/BRD kindly provided samples of LCT, and B. Somer supplied the samples of RT. Special thanks also go to B. Somer for providing us with much of the early CDFG literature on the stocking history and chemical/ electrofishing treatments of PCT populations. Funding support for this project was provided by the US Fish and Wildlife Service, Nevada Office.

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Received: 9 July 2003
Accepted: 6 February 2004

*California Fish and Game* 90(3):119-139 2004

# ORIGIN AND DISTRIBUTION OF LEOPARD FROGS, *RANA PIPIENS* COMPLEX, IN CALIFORNIA

MARK R. JENNINGS
Rana Resources
39913 Sharon Avenue
Davis, CA 95616-9456
and
Research Associate
Department of Herpetology
California Academy of Sciences
Golden Gate Park
San Francisco, CA 94118-4599

and

MICHAEL M. FULLER
Department of Biology
167A Castetter Hall
University of New Mexico
Albuquerque, NM 87131-1091

The distribution and status of ranid frogs, collectively known as "leopard frogs," *Rana pipiens* complex, in California is described. A review of museum specimens, historic field notes, and published literature—combined with field reconnaissance and interviews with local naturalists—confirms that the northern leopard frog, *R. pipiens*, and the lowland leopard frog, *R. yavapaiensis*, are native to the region east of the Sierra Nevada-Cascade crest and areas in the Colorado River basin, respectively. Early in the 20th Century, both species expanded their ranges into favorable habitats created by water diversions and large-scale irrigation projects. Since the 1970s, northern leopard frogs seem to have disappeared from most of their historic range and lowland leopard frogs appear to be extinct in the state. Northern leopard frogs of unknown origin were introduced into El Dorado, Kern, Los Angeles, Merced, San Francisco, Sierra, Tehama, and Tulare counties between 1905 and 1970. Several of these introduced populations experienced rapid growth and range expansions before completely disappearing. Except for a small population of northern leopard frogs present in Merced County, all of these introduced populations have apparently perished after persisting for varying periods of 5-25 years. The Rio Grande leopard frog, *R. berlandieri*, was accidentally introduced into the lower Colorado River in the late 1960s and has since spread rapidly in the Imperial Valley and agricultural areas along the lower Colorado River. The introduction and establishment of the southern leopard frog, *R. sphenocephala*, in a portion of the Santa Ana River basin of western Riverside and eastern Orange counties that has persisted since the late 1920s, is reported for the first time.

## INTRODUCTION

Over the past century, there has been considerable confusion regarding the status and genetic make-up of native and introduced populations of ranid frogs, collectively known as "leopard frogs," *Rana pipiens* complex, in California (Jennings 1983, 1987). Early publications by Cope (1889:405) and Dickerson (1906:172) indicate leopard frogs were found "between the eastern part of the Great Plains and Sierra Nevada Mountains." However, the first known California specimens of the northern leopard frog, *R. pipiens* (as defined in Pace 1974), were not collected until 20 July 1909 from Fallen Leaf Lake, El Dorado County, by the entomologist Francis Xavier Williams (CAS 14517-14519; see Table 1 for a key to museum symbolic codes). Between 1911 and 1916, dozens of northern leopard frogs were obtained at Tallac, El Dorado County, on the southwest shore of Lake Tahoe, and, again, at Fallen Leaf Lake (CAS 36326-36453, 39659-39660, CAS-SU 3489, 3502-3528, USNM 54967-54973), where they were abundant enough to provide food for mountain garter snakes, *Thamnophis elegans elegans* (Richardson 1915). Subsequently, Bryant (1917) reported that these northern leopard frogs had been introduced into the Lake Tahoe-Fallen Leaf Lake area about 1908, a statement that seemed accurate as only Sierra yellow-legged frogs, *R. sierrae*, had been collected in the Lake Tahoe region during earlier government scientific collecting expeditions in the 1870s (see Jennings 1984).

Table 1. Museum collections containing specimens of leopard frogs (*Rana pipiens* complex) collected in California. Museum symbolic codes follow Leviton et al. (1985) except for those marked with an asterisk (*).

| Code | Museum, Location |
|---|---|
| AMNH | American Museum of Natural History, New York, New York. |
| ASU | Arizona State University, Tempe, Arizona. |
| CAS | California Academy of Sciences, San Francisco, California. |
| CAS-SU | California Academy of Sciences-Stanford University Collection, San Francisco, California. |
| CSPU | California State Polytechnic University, Pomona, California. |
| CSUC* | Chico State University, Chico, California. |
| FRC* | Feather River College, Quincy, California. |
| HSU | Humboldt State University, Arcata, California. |
| KU | University of Kansas, Lawrence, Kansas. |
| LACM | Natural History Museum of Los Angeles County, Los Angeles, California. |
| MVZ | Museum of Vertebrate Zoology, University of California, Berkeley, California. |
| SDSNH | San Diego Natural History Museum, San Diego, California. |
| SSU | Sacramento State University, Sacramento, California. |
| UCD* | University of California, Davis, California. |
| UMMZ | Museum of Zoology, University of Michigan, Ann Arbor, Michigan. |
| USNM | National Museum of Natural History, Washington, D.C. |

In the lower Colorado River, leopard frogs (formerly referred to as *R. pipiens*) were apparently first observed near Fort Yuma[1], Imperial County, in February-March 1894 by Mearns (1907). The first known California specimen was collected 9.65 km west of Imperial, Imperial County, on 6 May 1909 by the zoologist Frank Stephens (MVZ 1058). All specimens obtained prior to 1966 from the lower Colorado River basin have now been identified as the lowland leopard frog, *R. yavapaiensis* (Platz and Frost 1984, Clarkson and Rorabaugh 1989). Despite some reservations expressed by Stebbins (1951, 1954, 1966, 1972), lowland leopard frogs are apparently native to the lower Colorado River (Van Denburgh and Slevin 1913), as well as natural overflow lakes and tributary streams in the Imperial Valley. This frog was known to be present at isolated locations such as Carrizo Wash, Harper's Well Wash, and Kane Springs west of the Salton Sea, Imperial County, prior to 1940 (MVZ 27187-27200, 27893-27897, 31619) (Ruibal 1959, 1962; Platz 1988). The natural presence of lowland leopard frogs at the latter three sites was questioned by the late George Sprague Myers and Laurence Monroe Klauber because they had not observed leopard frogs during their visits to this area in the late 1920s and early 1930s (R. R. Miller, unpublished field notes for 27 December 1938, UMMZ Archives). However, the lack of lowland leopard frog observations in western Imperial County by these capable field herpetologists in itself does not dispute a native origin. Conversely, other observations indicate lowland leopard frogs expanded their range in the Imperial Valley and along the Colorado River with the development of large-scale, irrigated agriculture in former desert areas during the early part of the 20th Century (Storer 1925, Klauber 1934).

Storer (1925) attempted to determine the status of all native and introduced leopard frog populations in California. Although he indicates that leopard frogs are believed to be introduced at Lake Tahoe and possibly at Alturas, Modoc County as well, his field notes indicate ambiguity regarding the status of northern leopard frogs in El Dorado and Modoc counties. For example, one resident of Alturas stated that "French frogs [=*R. pipiens*] were introduced into Surprise Valley about 24 years ago" (T. I. Storer, unpublished field notes for 30 August 1929, CAS Archives) while other long-time residents recalled that the frogs were native. More recent interviews with residents suggest that leopard frogs are native to the area (T. L. Rodgers, unpublished field notes for 24 July 1960). The uncertainty over the origin of leopard frog populations is reflected in Stebbins (1951, 1954, 1972) who used Storer's unpublished information to state that *R. pipiens* had been introduced into Lake Tahoe and Fallen Leaf Lake, and possibly Lake City and Alturas in Modoc County.

Bury and Luckenbach (1976) provided the most recent status review of leopard frog populations in California. Although they included information on several general locations in central and southern California where leopard frogs of unknown origin have been collected, no follow-up studies on the present status of these populations have been attempted. Further, there is some uncertainty regarding the specific identity

[1]Fort Yuma was apparently not considered to be officially located within the State of California until after official surveys during the mid-20th Century permanently affixed the present boundary line between California and Arizona.

of introduced leopard frogs discussed in Bury and Luckenbach (1976) because a number of taxonomic revisions have been published on the *R. pipiens* complex since 1973 (see the review by Hillis 1988). Additionally, Bury and Luckenbach (1976) felt that northern leopard frogs were probably introduced to the upper Owens Valley where they have been recorded since at least 1942 (UMMZ 91666, 91985). The recent finding of this species at an isolated locality on the east side of the White Mountains below Boundary Peak, Esmeralda County, Nevada, at 2,130 m elevation (Macey and Papenfuss 1991), provides compelling evidence that *R. pipiens* is native to the area.

In light of the uncertainty regarding the status of native and introduced leopard frog populations in the state, we believe it is important to document what is currently known about these populations in light of the precipitous decline of native ranid frog species over much of California (Jennings and Hayes 1994a, b; Jennings 1995) and other parts of the American West as well (Hayes and Jennings 1986, Stebbins and Cohen 1995). In this paper we discuss the fate of known introductions of northern leopard frogs; Rio Grande leopard frogs, *R. berlandieri*, and southern leopard frogs, *R. sphenocephala*,[2] into California over the past century. Additionally, we provide keys to aid in the proper identification of all leopard frog species currently known from the state.

## METHODS

Following the methodology of Jennings and Hayes (1994a), natural history collections, historic field notes, and the published literature were examined for records of leopard frogs from California. Field surveys using headlamps at night were performed at historic leopard frog sites to determine the present status of previously recorded populations. Additionally, local naturalists familiar with the frogs of California were interviewed (individuals are listed in the acknowledgments). Museum specimens from introduced leopard frog populations were carefully examined by one or both of us to confirm their identity. Sixteen natural history collections were found to contain leopard frog specimens collected in California (Table 1).

## RESULTS

A total of 516 museum specimens and 18 other records reveals that four species of leopard frogs are currently present in California. Northern leopard frogs are native to the upper Pit River basin, Shasta, Lassen, and Modoc counties; the Surprise Valley, Modoc County; the lower Klamath Lake basin, Siskiyou County; the Lake Tahoe region, El Dorado County; the Carson River drainage, Alpine County; and the Owens River Valley, Mono and Inyo counties. They have been introduced to a least 15 other locations in the state (Fig. 1). Lowland leopard frogs are native to the lower Colorado River basin and expanded their distribution early in the century with the advent of

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[2]The International Commission on Zoological Nomenclature (1992) has ruled that the correct scientific name for the southern leopard frog is *Rana sphenocephala* rather than *R. utricularius*.

irrigation in the Colorado and Imperial valleys (Fig. 2). Rio Grande leopard frogs and southern leopard frogs are 20th Century introductions that continue to expand their ranges (Fig. 3). A summary of known introductions for all leopard frog species follows.

Figure 1. Historic and current distribution of introduced (arrows) and presumed native populations of the northern leopard frog (*Rana pipiens*) in California based on 16 locations from 27 museum records and 5 records from other sources, and 43 locations from 340 museum records and 3 records from other sources, respectively.

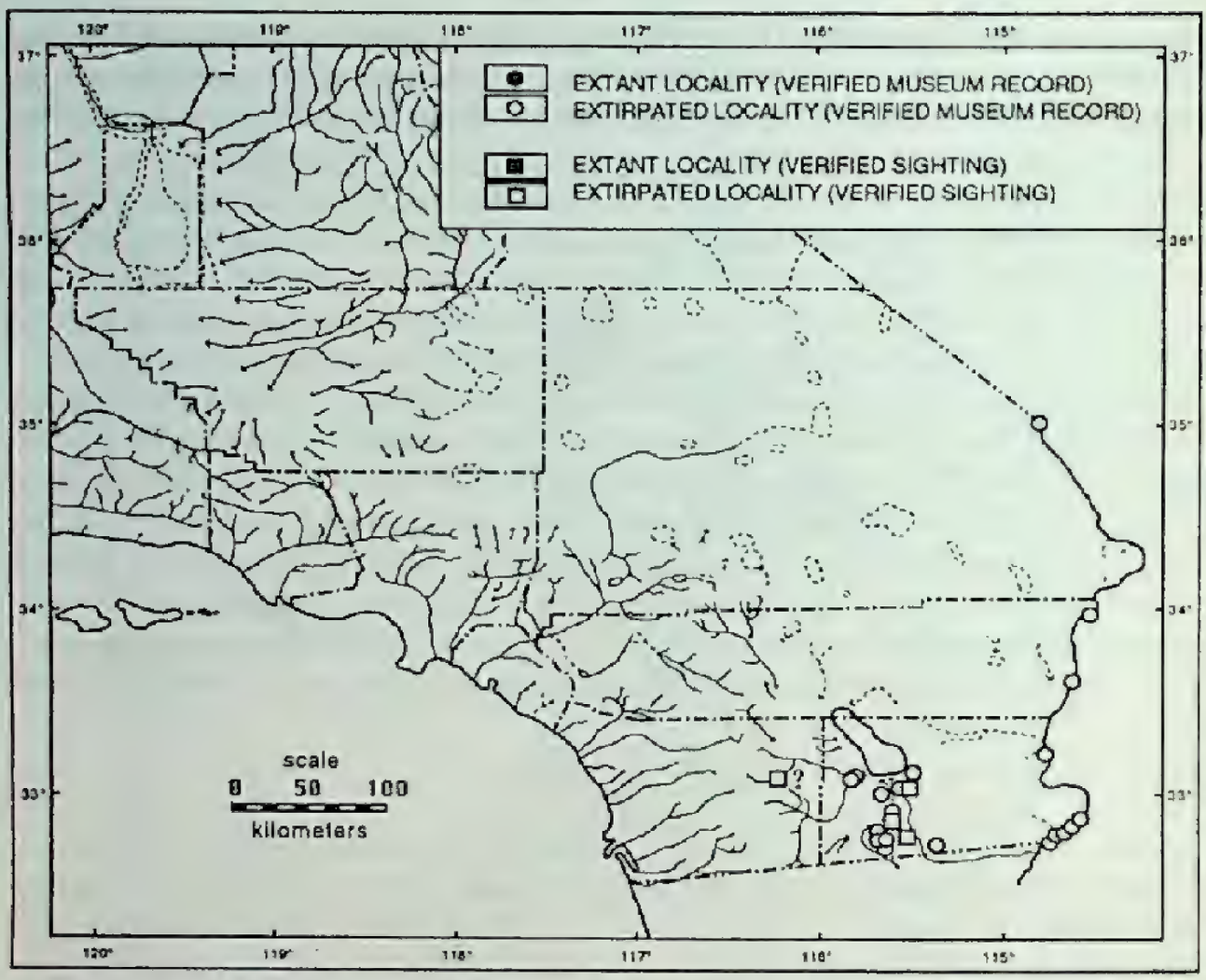


Figure 2. Historic and current distribution of the lowland leopard frog (*Rana yavapaiensis*) in southern California based on 28 locations from 116 museum records and 5 records from other sources.

## Northern Leopard Frog

According to Bryant (1917:90), northern leopard frogs were regularly seen in San Francisco fish markets and "there are constant attempts to introduce it into other suitable localities in the state." The only known introduction site mentioned by Bryant (1917) is at Tallac, where Melville Lawrence and Harry D. Comstock of the New Brockway Hotel and Hot Springs, Brockway, El Dorado County, California, transplanted 100 dozen leopard frogs collected from Carson Valley Reservoir, Douglas County, Nevada. Bryant (1917:90) recorded the date of transplantation as "about 8 years ago" which would place it around the year 1909. The Nevada collection site was reported to have been previously stocked with leopard frogs from somewhere else in Nevada (Bryant 1917). However, extreme western Nevada is within the native range of *R. pipiens* (Linsdale 1940, Pace 1974). The transplantation site in California is near the original state fish hatchery at the mouth of Taylor Creek on the southwest shore of Lake Tahoe (Leitritz 1970). Apparently the transplanted leopard frogs were intended to provide a ready source of fresh frog legs for the patrons of the hotel (Bryant 1917), as the Lake

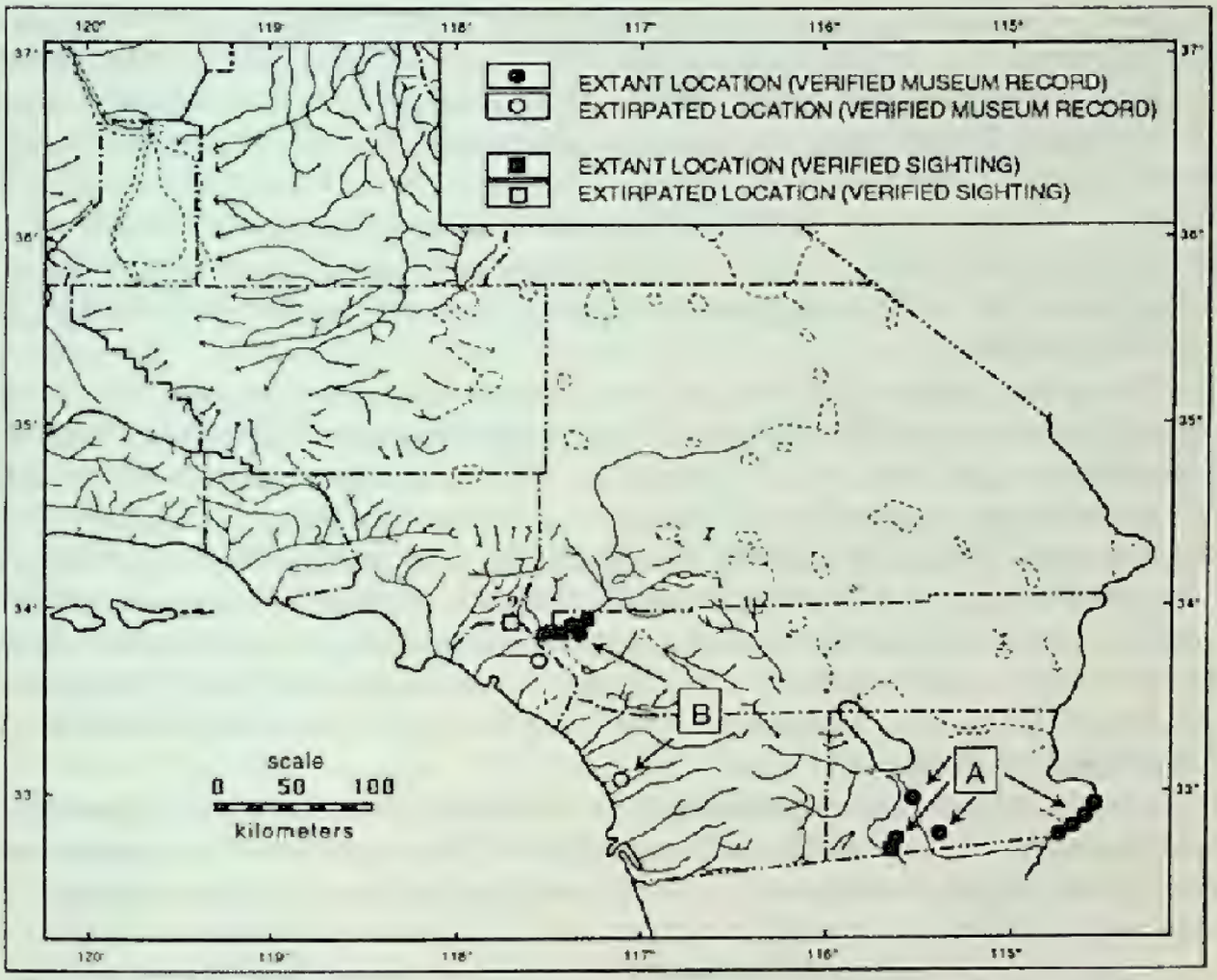


Figure 3. Historic and current distribution of: A) the introduced Rio Grande leopard frog (*Rana berlandieri*), and B) the introduced southern leopard frog (*R. sphenocephala*) in southern California based on 7 locations from 14 museum records and 3 records from other sources, and 9 locations from 19 museum records and 2 records from other sources respectively.

Tahoe area hotels owned by Lawrence and Comstock at the turn of the century were well-known throughout the United States for their fine cuisine (Scott 1957). In a 24 April 1924 letter from William H. Shebley to Tracy Irwin Storer (in Storer file "Alien Reptiles, Amphibians, Fishes Calif.", CAS Archives), Shebley states that the transplantation occurred about 1904 rather than 1909. Shebley was previously in charge of the state fish hatchery at Tallac (Leitritz 1970).

As mentioned earlier, the marshlands of the Tallac-Fallen Leaf Lake area have provided dozens of northern leopard frog specimens over the past 90 years. The last recorded specimens were collected on 23 August 1970 from Trout Creek at the confluence with Cold Creek, El Dorado County (MVZ 100316-100317). There have been no observations of leopard frogs in the entire Lake Tahoe basin during the past 2 decades and the species is presumed to be near extinction in the area (Jennings and Hayes 1994b, Jennings 1996).

Northern leopard frogs were also introduced on the Elliot and Hickman ranches east

of Red Bluff and at Battle Creek Meadows [=Mineral Post Office], Tehama County, in 1918 (Storer 1925). The frogs near Red Bluff were reported to be "thriving" in 1920, but could not be found in 1924 (Storer 1925). There are no additional records of leopard frogs from the Mineral area, although Cascade frogs, *Rana cascadae*, were found in the meadows in the vicinity of the old post office between 1924 and 1975 (MVZ 9982, 10001, 10008-10012, 10023, 10875-10879, 10881-10883; UCD 3603, 4172; G. E. Hansen, unpublished field notes [#77] for 24 July 1975). Subsequent surveys conducted during September, 1990 at Mineral found no Cascade frogs or leopard frogs (Jennings, unpublished data).

During the 1950s and 1960s, northern leopard frogs were reported from west-flowing streams in the Sierra Nevada (Bury and Luckenbach 1976). These records probably represent leopard frogs expanding their range westward or human-aided transplantations. One frog was collected on 15 August 1965 along the South Fork of the American River, 0.6 km west of Riverton, El Dorado County (SSU 542). No other frogs have since been observed at the site (J. M. Brode, California Department of Fish and Game, personal communication). Another frog was collected on 3 July 1958 from a small stream 1.6 km northeast of Sierraville, Sierra County (SSU 356). Subsequent surveys conducted during September, 1990 in the Sierraville area located no ranid frogs (Jennings, unpublished data).

A northern leopard frog specimen taken on 14 May 1963 from the campground at Old Station on Hat Creek, Shasta County (CSUC 778), is presumed to represent an isolated native population (Jennings and Hayes 1994b). This population is apparently now extinct (Jennings, unpublished data).

According to Banta and Morafka (1966), northern leopard frogs were frequently found in the rock garden ponds of Strybing Arboretum in Golden Gate Park, San Francisco County, during the 1950s. These frogs were most likely released pets or perhaps deliberately introduced by Arboretum personnel. Herbicides and the installation of a water filter system apparently exterminated the population by 1960 (Banta and Morafka 1966).

During June 1965, two adult male northern leopard frogs were collected on the East Side Canal near Fairfax Road and Edison Highway (east of Bakersfield), Kern County (MVZ 79415-79416). These frogs probably represent liberated pets or fishbait introductions. No other leopard frogs have been noted in this area, although bullfrogs, *R. catesbeiana*, are commonly observed there (Jennings, unpublished data).

There are several records for northern leopard frogs in the vicinity of Los Angeles, Los Angeles County. One specimen was collected during 1963 in Los Angeles (SSU 771) and another was collected from the East Fork of the San Gabriel River, San Gabriel Mountains on 28 April 1961 (CSPU 737). A third specimen, a juvenile collected on 24 August 1969 from Malibu Creek, 0.4 km south of Tapia Park, Santa Monica Mountains, represents the most recent known collection. These animals were presumably liberated pets or discarded laboratory frogs and probably served as the basis for Dixon (1967) reporting *R. pipiens* from the region.

Since that time, northern leopard frogs have not been observed in Malibu Creek or

the East Fork of the San Gabriel River despite intensive searches during the 1980s and 1990s (DeLisle et al. 1986; Jennings[3] 1994). Bullfrogs are now present in Malibu Creek and are spreading throughout the drainage (DeLisle et al. 1986). All ranids have now disappeared from the middle and lower portions of the East Fork of the San Gabriel River (Jennings[3] 1994).

Sometime during the 1960s or 1970s, northern leopard frogs were introduced into Owens Creek along Dan McNamera Road, about 1.6 km north of the Merced National Wildlife Refuge, Merced County. A single adult specimen (with a broken leg) was collected by George E. Hansen on 17 June 1976 (CAS 178498) from the creek where bullfrogs were also observed. Leopard frogs and bullfrogs still persist at the site, although the leopard frogs only inhabit Owens and nearby Bear creeks and have not successfully colonized the surrounding farmlands and irrigation ditches (G. E. Hansen, Sacramento, California, personal communication; Jennings, unpublished data).

During the 1950s, northern leopard frogs were apparently introduced into a location in extreme northwestern Tulare County. These leopard frogs spread from the original introduction site and colonized nearby irrigation canals. The first specimens were collected by Paul Umino at a location 3.2 km east of Orosi on 27 September 1958 and later donated by Robert G. Crippen to the Museum of Vertebrate Zoology in 1959 (MVZ 67764-67770). On 4 October 1961, Harold E. Basey collected specimens 3.2 km southwest and 0.3 km north of Yettem (MVZ 72630-72640). These collections contained metamorphs, juveniles, and adults and apparently provided the basis for later statements regarding the establishment of northern leopard frogs on the San Joaquin Valley floor in Tulare County (Stebbins 1966, 1972, 1985, 2003; Moyle 1973). Although the original source of these frogs is unknown, after their introduction they spread rapidly into nearby canals, Cross Creek, Cottonwood Creek, and the St. Johns River (FRC uncat.) where they built up populations of hundreds of individuals by the late 1960s (J. W. Wright, Feather River College, and H. E. Basey, Modesto Junior College, personal communication). Based the collection localities of museum specimens, the area eventually colonized by these frogs included at least 30 km of canals and streams.

Recent attempts to find northern leopard frogs at sites were they once occurred in Tulare County have only disclosed the presence of bullfrogs (Jennings, unpublished data). Many of the old farm ponds and well ponds that once held water for nearby crops, vineyards, and orchards have been filled, or covered over with soil, due to changing farming practices. Additionally, there is more efficient use of water in irrigation canals and related drops in water depth may have led to the demise of introduced northern leopard frog populations.

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[3]Jennings, M. R. 1994. Status of aquatic amphibians in the Sheep Mountain Wilderness Area, Angeles National Forest. Final report prepared for the United States Department of Agriculture, Angeles National Forest, Arcadia, California, through Interagency Agreement (5-01-69-019). National Biological Service, California Pacific Science Center, Piedras Blancas Research Station, San Simeon, California.

## Rio Grande Leopard Frog

Rio Grande leopard frogs seem to have been accidentally introduced into the lower Colorado River near Yuma, Arizona, via one or more fish plants from either Texas or New Mexico between 1965 and 1971 (Platz et al. 1990). Since 1981, the species has expanded its range and has been taken in agricultural areas along the lower Colorado River (Clarkson and Rorabaugh 1989) and in the Imperial Valley (Jennings and Hayes 1994a). The region colonized by this frog is within the historic range of the native lowland leopard frog which apparently disappeared from California in the 1970s (Jennings and Hayes 1994a). More recent observations indicate that the Rio Grande leopard frog continues to expand its range in the Imperial Valley (Fig. 3). It has been collected from several areas in and around El Centro, Imperial County (Jennings and Hayes 1994a; CAS 210844-210846), as well as the Hot Spring Long-Term Visitor Area (next to the East Highline Canal), Imperial County (CAS 216722-216724), and at more than a dozen other locations in the Imperial Valley that have been intensively surveyed during the past 2 years (J. C. Rorabaugh, unpublished data). Rio Grande leopard frogs will probably be found at other locations (especially along the All American Canal) in Imperial County in the near future. In almost all locations noted, Rio Grande leopard frogs have been observed in association with bullfrogs (Jennings and Hayes 1994a).

## Southern Leopard Frog

Southern leopard frogs in California were first recorded as "*R. pipiens*" at the Chino Gun Club, Riverside County, about 1929 or 1930 (1 August 1986 letter from C. M. Bogert to Jennings). This record is from a tributary to the Santa Ana River, about 4.83 km west of Corona, Riverside County (within the present area of the Prado Flood Control Basin). In an 8 February 1937 letter from Bogert to Storer (in Storer file "Alien Reptiles, Amphibians, Fishes Calif.", CAS Archives), Bogert mentioned that he had collected and identified two leopard frog specimens and had observed other leopard frogs on the Club properties. Unfortunately, the two leopard frog specimens were apparently not saved. Although the source of this leopard frog population was not determined by Bogert, we speculate that it originated from Louisiana (which is within the historic range of *R. sphenocephala*) because the Chino Gun Club imported bullfrogs; mosquitofish, *Gambusia affinis*; and Louisiana red swamp crayfish, *Procambarus clarkii*, for their properties during the 1920s from dealers in Louisiana (see Finley 1932). The southern leopard frogs were most likely introduced as larvae along with the mosquitofish or crayfish.

Since the original introduction to the Chino Gun Club properties, southern leopard frogs have expanded their range within the Santa Ana River Basin in eastern Orange and western Riverside counties (Fig. 3). Juvenile and adult specimens have been collected from various points along the Santa Ana River from the Anaheim Hills, Orange County, to Corona, Riverside County, as well as the southwest corner of Irvine Lake, Orange County, in 1957, 1961, 1963, and 1967 (LACM 1975, 91314-91327). It is unknown if the frogs identified as *R. pipiens* from nearby Tonner Canyon in Los Angeles and

Orange counties (Vincent[4] 1967) were actually southern leopard frogs. This latter location was thoroughly checked during April and August, 1994, but no leopard frogs were found; bullfrogs were observed (Fuller, unpublished data; 19 September 1994 letter from G. R. Stewart to Jennings).

Southern leopard frogs are presently common in the Prado Flood Control Basin. Recent collections along Temescal Creek just northeast of the Corona Municipal Airport, Riverside County, reveals that they occur in areas that are rapidly becoming urbanized (CAS 197588, 197603). Additionally, these frogs share their habitat with two other introduced amphibians—bullfrogs and African clawed frogs (*Xenopus laevis*) (Fuller, unpublished data).

An additional specimen of the southern leopard frog was collected from Olivehain Pond (a small reservoir just west of Olivehain), San Diego County, on 10 March 1957 (LACM 91328). The origin of this animal is unknown and it probably represents a released pet or fishbait introduction. The entire Olivehain area was urbanized between 1968 and 1983 and there are no further records of leopard frogs from this area (Jennings, unpublished data).

## DISCUSSION

Although there have been many attempts to introduce various leopard frog species into California, most introductions were unsuccessful. The most common factors resulting in these introductions are the use of live leopard frogs as fishbait and the inadvertent introduction of larvae via fish plants. Leopard frogs are also commonly sold in the pet trade and as laboratory animals in high schools and colleges and there are a number of examples of these animals being liberated by well-meaning, but naive, individuals, especially in parks or wildlife refuges near urban areas (Dixon 1967; Jennings, unpublished data).

In the few cases where introduced leopard frogs have managed to establish reproducing populations, they seem to do best in human-altered environments such as irrigation canals and artificial ponds with other introduced fishes and amphibians. When leopard frogs do become established, they tend to build up large populations that expand into surrounding aquatic areas. This pattern was observed with Rio Grande leopard frogs along the lower Colorado River, southern leopard frogs in the Santa Ana River, and northern leopard frogs in the San Joaquin Valley.

Introduced leopard frogs have the potential to negatively affect our native herpetofauna. Already, the Rio Grande leopard frog has apparently replaced the lowland leopard frog along the lower Colorado River and in the Imperial Valley (Jennings and Hayes 1994a) and there is a possibility that if introduced stocks of montane-adapted leopard frogs become established, they might replace native species such as the Cascade frog, Sierra yellow-legged frog, and spotted frog, *R. pretiosa* (Bury

[4]Vincent, J. G. 1967. An ecological study of Tonner Canyon. Unpublished M.S. Thesis, Whittier College, Whittier, California.

and Luckenbach 1976), species which have greatly declined in distribution during the past 2 decades (Jennings and Hayes 1994b, Jennings 1995). Indeed, northern leopard frogs have replaced spotted frogs in Idaho and eastern Oregon during the 1960s and 1970s (Dumas 1964, 1966; Nussbaum et al. 1983).

In light of the many leopard frog introductions into California over the past 90 years, efforts should be made to discourage the use of live leopard frogs for fishbait and improve public education to curb the release into the wild of unwanted pet and laboratory leopard frogs. One way to achieve this goal is through appropriate statements in sport fishing regulations and natural resources publications aimed at the general public. Other options include presentations in K-12 educational programs and the posting of educational posters in pet stores, at reptile fairs, and at urban wildlife refuges. Although little if anything can be done to prevent the spread of currently established introduced leopard frog populations in the wild, we believe it is important to document the spread of these populations at regular intervals and take any steps available to lessen the possibility of future introductions.

Because of uncertainty regarding the identification of leopard frogs that might be encountered in California, we provide the following key, which is modified from information presented in Hillis (1982), Scott and Jennings (1985, 1989), and Conant and Collins (1998). We caution that anyone attempting larval stage identifications should have the specimens verified by an expert. We additionally suggest the use of an adequate field guide (e.g., Smith 1978, Behler and King 1979, Stebbins 2003) to assist in distinguishing the differences between leopard frogs and other native and introduced frogs. We strongly urge that anyone finding a leopard frog west of the Sierra Nevada-Cascade crest submit the animal to the proper authorities to be preserved as a voucher specimen for future reference.

## Key to the Leopard Frogs Inhabiting California

Postmetamorphic stages (>40 mm snout-vent length)—

1a. Frog without a dorsolateral fold (Fig. 4a) [bullfrog (*R. catesbeiana*)].

1b. Frog with a partial or incomplete dorsolateral fold (Fig. 4b) [foothill yellow-legged frog, *R. boylii*, Sierra yellow-legged frog, *R. sierrae*, and Transverse Range yellow-legged frog, *R. muscosa*].

1c. Frog with a complete (Fig. 4c), or almost complete (Fig. 4d), dorsolateral fold.....see 2.

2a. Frog with dorsal spots or blotches that contain light-colored centers (Fig. 5a) or inky black spots (Fig. 5b) [red-legged frog, *R. aurora*, Cascade frog, *R. cascadae*, and spotted frog, *R. pretiosa*].

2b. Frog with solid black dorsal spots or blotches surrounded by light-colored boarders (Figs. 5c and 5d).....see 3.

3a. Frog with unbroken dorsolateral folds (Fig. 4c).....see 4.

3b. Frog with broken dorsolateral folds (Fig. 4d).....see 5.

4a. Frog with blunt snout, many spots below dorsolateral folds, and discrete spots on rear of thighs (Fig. 5c) [northern leopard frog, *R. pipiens*].

4b. Frog with elongate snout, few spots below dorsolateral folds, and a patchwork of fine spots on rear of thighs (Fig. 5d) [southern leopard frog, *R. sphenocephala*].

5a. Frog with large eyes and a well-connected network of large spots on rear of thigh (Fig. 6a) [Rio Grande leopard frog, *R. berlandieri*].

5b. Frog with medium eyes and a weakly-connected network of spots on rear of thigh (Fig. 6b) [lowland leopard frog, *R. yavapaiensis*].

Larval stages (>30 mm total length)—

1a. Tadpole with 4 or more lower tooth rows (Fig. 7a) [foothill yellow-legged frog, *R. boylii*, Cascade frog, *R. cascadae*, Sierra yellow-legged frog, *R. sierrae*, and Transverse Range yellow-legged frog, *R. muscosa*].

1b. Tadpole with 3 lower tooth rows (Fig. 7b).....see 2.

2a. Tadpole body and tail covered with distinct, discrete, black dots <1 mm in diameter; lateral line pores not visible to the naked eye (Fig. 8a) [bullfrog, *R. catesbeiana*].

2b. Tadpole body with large black splotches >1 mm in diameter; lateral line pores usually visible to the naked eye (Fig. 8b) [red-legged frog, *R. aurora*, and spotted frog, *R. pretiosa*].

2c. Tadpole body and tail with blotches or mottling that are not evenly distributed (Fig. 8c).....see 3.

3a. Tadpole with discrete dark spots or mottling on tail, including dorsal and ventral tail fins (Fig. 9a).....see 4.

3b. Tadpole with light mottling on tail, including dorsal and ventral tail fins (Fig. 9b).....see 5.

4a. Tadpole with discrete dark spots on head and body, and an apparent "v"-shaped lateral line system on the dorsum of the body (Fig. 10a) [lowland leopard frog, *R. yavapaiensis*].

4b. Tadpole with dark mottling on head and body, and a simple lateral line system on the dorsum of the body (Fig. 10b) [Rio Grande leopard frog, *R. berlandieri*].

5a. Tadpole with narrow tail muscles and a course network of mottling (Fig. 11a) [northern leopard frog, *R. pipiens*].

5b. Tadpole with wide tail muscles and a fine network of mottling (Fig. 11b) [southern leopard frog, *R. sphenocephala*].

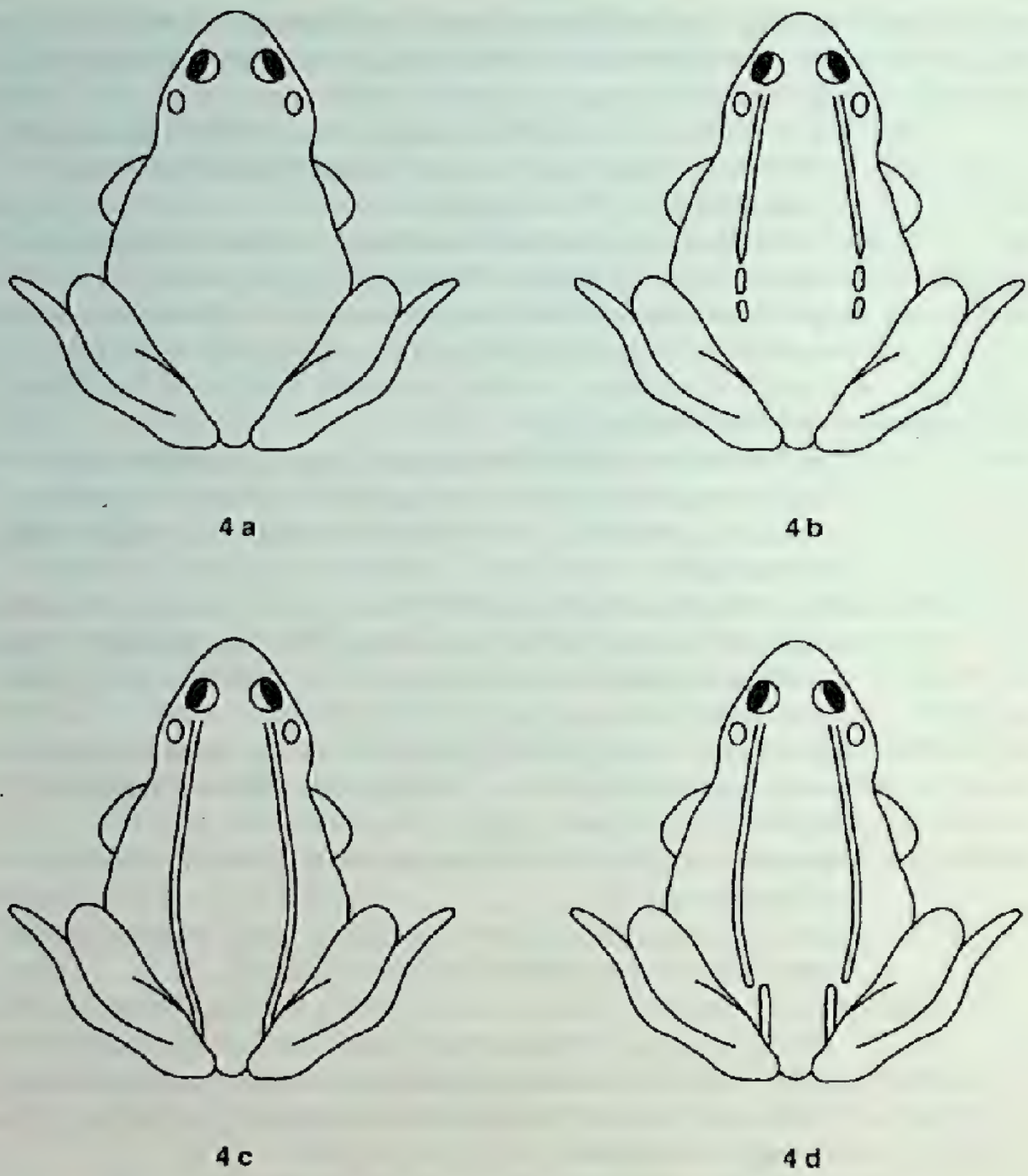
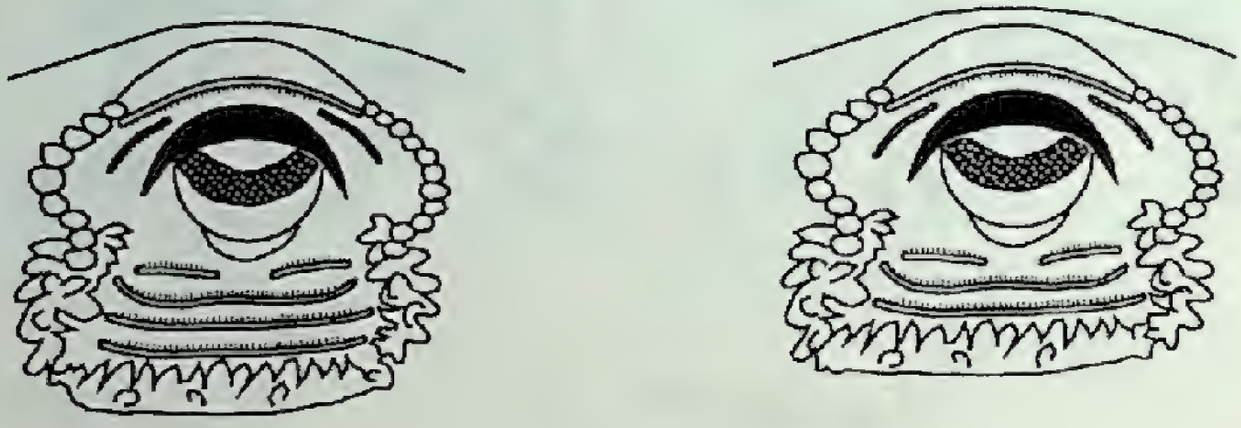



Figure 4. Characteristics of frogs lacking a dorsolateral fold (4a), having an incomplete dorsolateral fold (4b), having a complete dorsolateral ford (4c), and having an almost complete dorsolateral fold (4d).

Figure 5. Characteristics of dorsal spots that contain light-colored centers (5a), inky black spots (5b), and solid black dorsal spots (5c, northern leopard frog; 5d southern leopard frog).

6 a 6 b

Figure 6. Characteristics of the Rio Grande leopard frog (6a) and the lowland leopard frog (6b).



7 a 7 b

Figure 7. Frog tadpole mouthparts with 4 lower tooth rows (7a) and 3 lower tooth rows (7b).

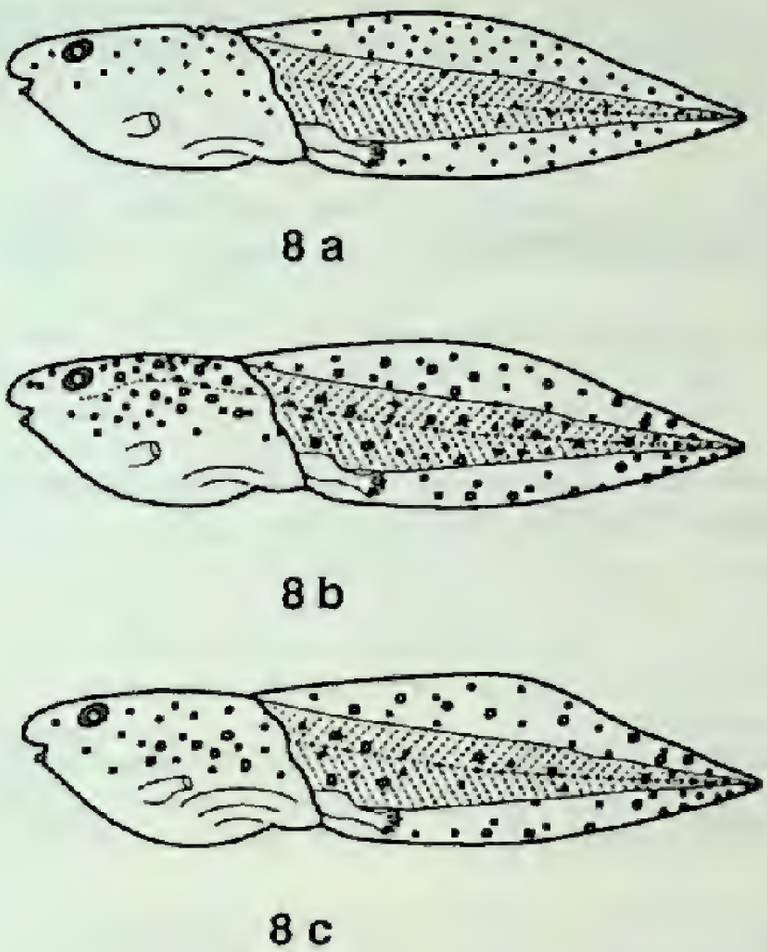



Figure 8. Side characteristics of tadpoles with black dots and no visible lateral line pores (8a), black splotches and visible lateral line pores (8b), and unevenly distributed mottling (8c).

Figure 9. Side characteristics of tadpoles with dark spotting (9a) and light spotting (9b).

Figure 10. Side and dorsal characteristics of tadpoles of the lowland leopard frog (10a) and the Rio Grande leopard frog (10b).

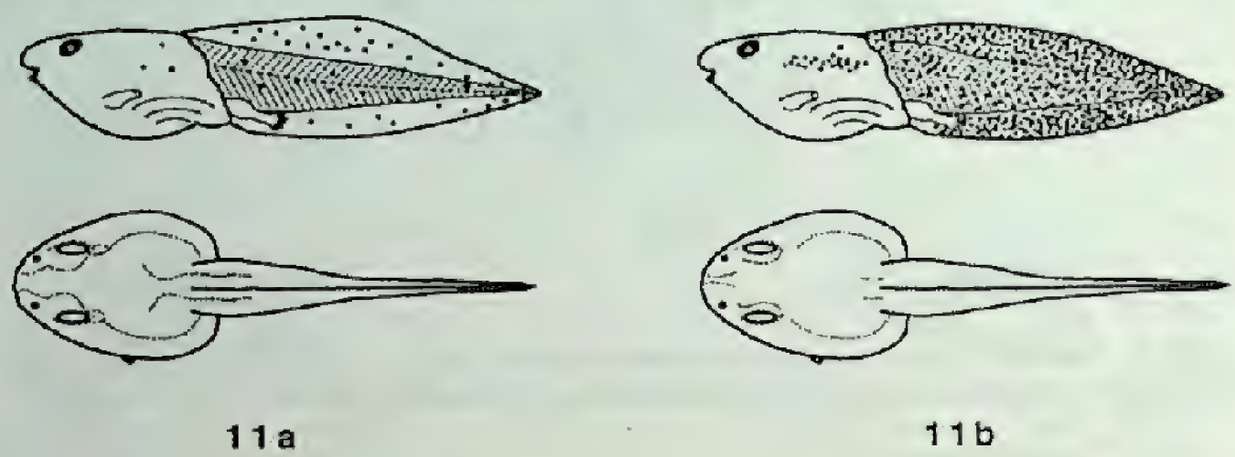



Figure 11. Side and dorsal characteristics of tadpoles of the northern leopard frog (11a) and the southern leopard frog (11b).

## ACKNOWLEDGMENTS

Our thanks to the following museum curators and personnel for their assistance and for allowing us to examine specimens under their care: J. E. Berrian, R. L. Bezy, the late C. M. Bogert, R. J. Bogiatto, M. R. Brittan, J. M. Brode, W. E. Duellman, D. R. Frost, D. A. Good, A. G. Kluge, G. K. Pregill, G. E. Schneider, H. B. Shaffer, J. E. Simmons, B. R. Stein, G. R. Stewart, J. V. Vindum, D. B. Wake, J. F. Waters, and J. W. (Jay) Wright. We also thank the following individuals for allowing us to copy field notes and other important archival information under their care: P. Ahlstrand, C. Barsi, H. E. Basey, J. M. Brode, T. R. Burkhardt, the late G. E. Hansen, J. C. Kooy, D. W. Nelson, the late T. L. Rodgers, J. C. Rorabaugh, B. R. Stein, and D. B. Wake. S. Fuller, R. Goodman, and M. P. Hayes assisted in the field and N. J. Scott, Jr., M. J. Sredl, and G. R. Stewart reviewed

the manuscript. Permits to collect specimens cited in this paper were provided to the authors by the California Department of Fish and Game.

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Received: 3 November 19[illegible]
Accepted: 31 March 200[illegible]

*California Fish and Game* 90(3):140-154 2004

# CURRENT STATUS OF SOUTHERN STEELHEAD/ RAINBOW TROUT IN SAN MATEO CREEK, CALIFORNIA

TIM E. HOVEY
California Department of Fish and Game
4949 Viewridge Avenue
San Diego, CA 92123

**The discovery of a small population (<70 individuals) of southern steelhead trout, *Oncorhynchus mykiss* in San Mateo Creek (SMC) and Devil Canyon Creek (DCC), a small tributary to SMC, in San Diego County, California in the spring 1999 represents the first multiple record account of trout in the creek in over 50 years. From August 1999 to December 2003, the California Department of Fish and Game (Department) conducted a total of 45 surveys along both creeks to monitor the status of the trout. Data on trout abundance, age analysis, water temperature, spawning behavior and genetic analysis were collected during the surveys. We observed that trout numbers on SMC began to decline shortly after the surveys began in 1999, and that the last trout was observed in August 2000. Juvenile trout were discovered on DCC in May and July 2000 and maturing fish were continually monitored on DCC through 2001 and 2002, with no new recruitment recorded during that time. A single adult holdover trout (DC2) was observed on the confluence as recently as December of 2003. Water temperature data collected on both creeks suggested that annual temperature profiles are more stable and suitable for southern steelhead survival in DCC than in SMC. We assume that this difference enhances trout survival in DCC. Spawning behavior was described on two different occasions on DCC, indicating the potential for resident reproduction. However, no actual spawning was ever recorded on SMC or DCC. Genetic and age analysis on specimens collected in 1999 and 2000 established that at least two pairs of anadromous steelhead entered the drainage in 1997 to spawn and that successful, resident reproduction (F1) occurred in DCC in 2000. Otolith age analysis of a single adult individual (DC1) collected in DCC in 2002 confirmed that maturing, second generation trout (F2) occupied that tributary, albeit in extremely small numbers (< 8 individuals). The numbers on DCC continued to drop until only a single adult trout (337 mm TL) was observed during a survey in December 2003. The higher water temperature and presence of non-native fish species likely contributed to the extirpation of adult trout on SMC in 2000. Conversely, the lack of non-native fish and the more stable water temperature in DCC enabled trout to survive on the tributary until the low water conditions impacted their numbers considerably.**

## INTRODUCTION

Southern steelhead trout historically occurred from Santo Domingo River in northern Baja California to Malibu Creek, California. Historic records also show that in

the early 1900's, San Mateo Creek may have been one of the most important southern steelhead spawning streams on the south coast (USFWS Report 1998[1]). At their peak, the San Mateo Creek steelhead runs were recorded as large and consistent, with the fish documented to attain a larger size than their relatives to the north. Despite the high numbers recorded in the early part of the Century, trout sightings dropped off in the 1940's and the 1950's and consistent trout abundance has not been present within San Mateo Creek or south of Malibu Creek in the last 50 years (USFWS Report 1995[2]). The last recorded sighting of multiple age-classes of southern steelhead on SMC was in 1950 during a Department stream survey. Since then, only anecdotal observations of trout on the creek have been recorded.

Since that time, inconsistent water availability due to an increase in ground water pumping beginning in the late 1950's and the negative influence of nonnative plants, (*Tamarisk* sp.), began to contribute to the alteration of stream habitat within SMC. Critical stream structures for trout reproduction, such as riffles and gravel beds now sit dry and useless due to altered flow dynamics. Inconsistent water levels also reduces the amount of time SMC remains open to the ocean, further limiting upstream migration of southern steelhead, as well as downstream emigration of resident fish.

Further effecting trout presence was the introduction of nonnative terrestrial and aquatic species (largemouth bass, *Micropterus salmoides*, green sunfish, *Lepomis cyanellus*, bluegill, *Lepomis macrochirus*, black bullhead, *Ameiurus melas*, mosquito fish, *Gambusia affinis*, swamp crayfish, *Procambarus clarkii*, and bullfrog, *Rana catesbeiana*) into San Mateo Creek. These species have been documented to increase competitive and predatory impacts on seasonal salmonid runs, as well as resident trout populations. Steelhead that were fortunate enough to find periodic available water to migrate upstream, often found their spawning grounds infested with nonnative fish species, heavily impacting reproductive success.

Due to these two factors (altered habitat and the introduction of aquatic, nonnative species), it was generally concluded that southern steelhead trout were extinct south of Malibu Creek, California.

The decline in quality, southern steelhead habitat and the absence of steelhead trout in southern California, prompted the National Marine Fisheries Service (NMFS) to list southern California's Evolutionarily Significant Unit (ESU) for anadromous steelhead as an endangered species in 1997 (Federal Registry 62 FR 43937). This listing, however, designated the current southern ESU boundary as Malibu Creek, California and protection was excluded for all potential waterways south of that boundary.

The discovery of southern steelhead/rainbow trout, *Oncorhynchus mykiss*, on SMC and Devil Canyon Creek (DCC) a small tributary to SMC, in San Diego County,

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[1]U.S. Fish and Wildlife Service. 1998, Southern Steelhead Oncorhynchus mykiss Habitat Suitability survey of the Santa Margarita River, San Mateo and San Onofre Creeks on Marine Corps Base, Camp Pendleton. Coastal California Fish and Wildlife Office, Arcata, CA. Prepared for Assistant Chief of Staff, Environmental Security.

[2]U.S. Fish and Wildlife Service. 1995, Santa Margarita River and San Mateo Creek Salmonid Study. Progress Report prepared for Camp Pendleton Marine Corps. Base, January-September 1995. Coastal California Fish and Wildlife Office. Arcata, CA.

California during spring 1999, established a need to extend the newly designated ESU boundary. The population size, though never accurately determined, was extremely small (<70 individuals), which limited the number of samples that could be collected (10 adults in 1999 and 5 juveniles in 2000) for analysis. Despite this, successful analysis was conducted on genetics, age, and otolith microchemistry for the SMC population.

Genetic analysis conducted on a fin-clip collected from a single adult in 1999 (SM412199F) revealed that the specimen carried a unique haplotype that is most commonly found in southern California steelhead and has never been seen in any hatchery population of steelhead or rainbow trout (Nielsen et al. 1994). This information confirmed that the trout occupying SMC were wild fish. Otolith microchemistry performed on a single specimen collected from SMC in 1999 confirmed that the examined fish was the progeny of an anadromous female steelhead (CDFG steelhead report, 2000[3]; Rieman et al. 1994) and that the cohorts occupying SMC were the offspring of ocean-going adults. Age analysis of a single otolith indicated that the fish were 2+ years of age when collected in 1999. Back calculating from the age at discovery, it was confirmed that the anadromous steelhead adults most likely entered the San Mateo drainage to spawn during a high water period in early spring 1997.

This information and additional monitoring observations were combined and submitted as an original report to the National Marine Fisheries Service (NMFS) by the California Department of Fish and Game (Department) in February 2000. It was hoped that this recent information on the newly discovered population of southern steelhead trout in SMC would require NMFS to re-evaluate the recently designated geographic boundary of the evolutionary significant unit (ESU) for this species. Due to the rarity of this occurrence, the Department also initiated a monitoring program to track the status of the steelhead/rainbow trout population on SMC immediately following the 1999 discovery. Continued monitoring began in August of 1999 and over the following 4-year period, additional information on genetics, age at growth, trout behavior, temperature conditions and population status was collected.

The objectives of this study are to: 1) describe the monitoring conducted and data gathered on the drainage since the re-discovery in 1999; and 2) to illustrate the factors affecting the survival and current status of the southern steelhead/rainbow trout in San Mateo and Devil Canyon creeks.

## METHODS AND MATERIALS

A total of 45 stream surveys were conducted by the Department from August 1999 to December 2003. Surveys were conducted by Department personnel and volunteers, and started at the United States Geological Survey (USGS No. 11046300) gauging station on SMC to the Devil Canyon Creek confluence and included the entire Devil Canyon Creek drainage. Three large pools located on SMC, Pools 1, 2 and 3, (Fig. 1)

[3]California Department of Fish and Game (CDFG) 2000. Steelhead rainbow trout in San Mateo Creek, San Diego County, California. Report submitted to the National Marine Fisheries Services. 22 pp.

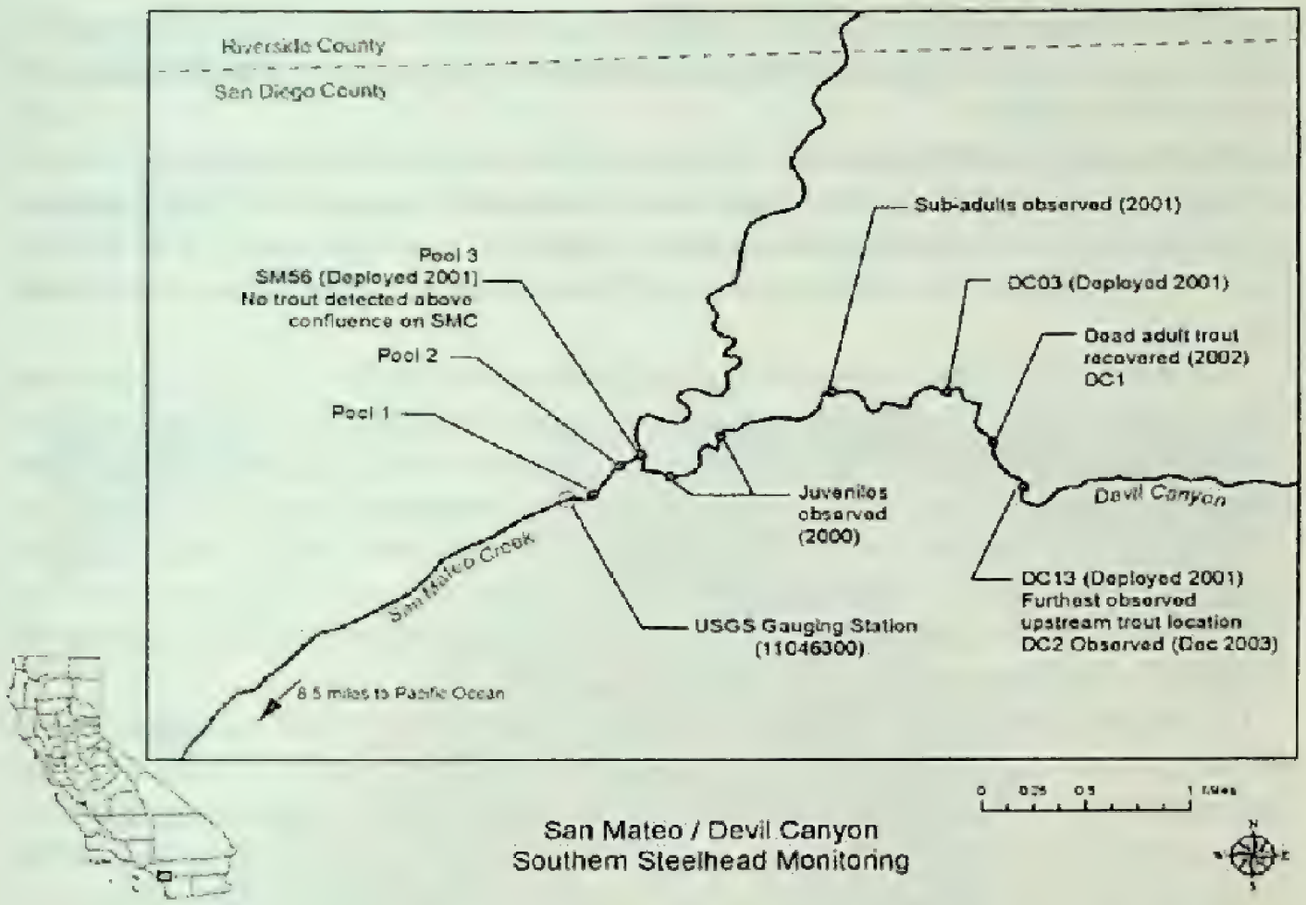


Figure 1. Map of survey area showing Pool 1, 2 and 3, temperature probe deployment areas, gauging station, and trout locations.

remained isolated for much of the survey period and was examined during each department survey. An additional 3.4 km of San Mateo Creek above the Devil Canyon Creek confluence were infrequently surveyed.

Survey techniques used were drainage mapping, back pack electro-shocking (Smith-Root 12-B), snorkeling, seining, and passive observation.

Drainage mapping involved hiking the DCC drainage to evaluate habitat and to identify individual trout locations. Data was collected on fish barriers, quality of habitat, and fish presence.

Electro-shocking surveys were conducted for exotic species removal and juvenile trout detection in areas where seining was not possible. Data were collected on species observed, water temperature, and effort (seconds) for each electro-shocking survey. The use of the back pack electro-shocker was limited during the survey period to reduce the possibility of injury to resident trout.

Presence/absence snorkel surveys were conducted in pooled areas where effective seining was difficult and where electro-shocking was ineffective. Snorkel surveys were conducted by a single observer using an underwater dive light. Each survey covered the entire pool and all fish species were recorded. When trout were observed, size (total length) was estimated and over all health assessed.

Two different types of seines were used to sample the drainage: a 3-m beach seine and a 12-m pole seine. Seines were used for exotic species removal and to determine if trout were present.

Passive presence/absence shore observations were conducted on several occasions to record trout sightings and abundance. Data were collected on the length of observation and the species encountered. All trout sightings obtained by passive shore observations were visually confirmed with follow-up snorkel surveys the same day.

A total of 15 fin-clips were collected for genetic purposes from March 1999 to June 2000 (Table 1). Each sample comprised of a 1-cm square piece of fin tissue excised from the upper lobe of the caudal fin and placed on filter paper to dry. All dried samples were placed in small envelopes for analysis. A single genetic sample was sent for mitochondrial DNA (mtDNA) analysis in 1999 and verified the resident trout as wild fish. This specimen is designated as SM412199F in Table 1. The remaining 14 samples were sent out for mitochondrial DNA (mtDNA) analysis in March of 2001 and we report on the findings of that analysis in this study (Table 1).

Three temperature loggers (two Onset Optic StowAway model number WTA08-05+37 and one Onset Optic StowAway Tidbit model number TB132-05+37) were deployed (two on DCC and one on SMC) in August 2001. Each probe gathered temperature data every 15 minutes for a period of over 11 months. Data logger No. 471213 (DC13) was deployed in a small pool at GPS N 33.47665, W 117.45485 on DCC, approximately 1.6 km from SMC (Fig. 1). Tidbit data logger No. 364203 (DC03) was

Table 1. Trout genetic samples collected on San Mateo Creek and Devil Canyon Creek

| Sample | Date Collected | Length mm TL | Collection Location | Date Analyzed |
|---|---|---|---|---|
| SM412199F | 4/21/99 | 217 | Devil Canyon | May 1999 |
| SM1F | 5/13/99 | 204 | San Mateo Creek | March 2001 |
| SM2F | 5/13/99 | 202 | San Mateo Creek | March 2001 |
| SM3F | 5/13/99 | 245 | Devil Canyon | March 2001 |
| SM4F | 5/13/99 | 233 | San Mateo Creek | March 2001 |
| SM5F | 5/13/99 | 198 | San Mateo Creek | March 2001 |
| SM6F | 5/13/99 | 196 | San Mateo Creek | March 2001 |
| SM7F | 5/13/99 | 193 | San Mateo Creek | March 2001 |
| SM8F | 5/13/99 | 164 | San Mateo Creek | March 2001 |
| SM11F | 5/13/99 | 180 | San Mateo Creek | March 2001 |
| SM15F | 5/26/00 | 60 | Devil Canyon | March 2001 |
| SM16F | 5/26/00 | 65 | Devil Canyon | March 2001 |
| SM17F | 7/7/00 | 81 | Devil Canyon | March 2001 |
| SM18F | 7/7/00 | 74 | Devil Canyon | March 2001 |
| SM19F | 7/7/00 | 60 | Devil Canyon | March 2001 |

deployed in a larger pool at GPS N 33.47453, W 117.45926 on DCC, approximately 0.8 km from SMC (Fig. 1). Data logger No. 471256 (SM56) was deployed in Pool 3 on SMC (Fig. 1).

A single adult trout was collected in June 2002 on DCC (designated DC1) and examined to determine age. The fish was weighed, measured, and dissected. The sagittal otoliths were removed, cleaned, and age determination, using a dissecting scope, was confirmed by four readers (Fig. 2).

## RESULTS

Figure 2. Otolith micrograph of trout collected on Devil Canyon Creek in June 2002. Age was determined to be 2 + years of age. Specimen was 273 mm TL. Photo by R. Titus

Observations during the survey period (August 1999 to December 2003) revealed that the trout located in SMC declined in numbers, with no trout being observed after August 2000. In May and July 2000, a total of 22 juvenile trout, ranging in size from 60-81 mm TL, was discovered occupying the first 1.2 km of DCC. Continued observations of juvenile trout on DCC lasted through September 2000. No juvenile trout were located in SMC during the survey period. In August 2001, several adult trout (200-250 mm TL) were observed in two separate pools on DCC, approximately 0.8 and 1.6 km from the SMC confluence (Fig. 1). These were assumed to be cohorts of the juveniles found in 2000. Observations of adults occupying DCC approximately 1.6 km up from the confluence continued through December 2003. Trout were frequently observed in DCC occupying pools during the spring where they were previously absent, indicating possible downstream migration.

While the age at size of the juveniles found on DCC could not be confirmed, the measurements of the individuals are well within the maximum first year growth rate of size-at-time data collected by the Department of known-age juvenile steelhead trout in south-central and southern California coast (CDFG Steelhead Report 2000).

Reproductive activity was observed on DCC in August 2001 and January 2002. Three adult trout were observed participating in brief spawning episodes on Devil Canyon Creek, in a pool approximately 0.8 km from the San Mateo Creek confluence. Individuals were using a central boulder as a staging area and conducting reproductive posturing directly upstream of the boulder. A redd had been constructed adjacent to the boulder and individuals were observed approaching the redd and rubbing themselves on the gravel bottom. The redd was approximately 45-60 cm in diameter and appeared as a circular, sandy area where all the leaf litter had been either removed or covered. One observation was recorded while the pool was being snorkeled and another was recorded from a rock outcropping directly above the pool. Both episodes were brief and activity ceased once the observers were detected by the trout. While this was encouraging, no actual spawning was observed and no new recruitment was documented for 2001 or 2002.

Mitochondrial DNA (mtDNA) was successfully extracted from all fin-clip specimens and analyzed according to established analytical methods (Nielsen et al. 1997). Five of the 1999 samples (SM3, SM4, SM7, SM8, and SM11) showed a lack of differentiation between the trout collected, indicating that these fish were cohorts of the previously analyzed SM412199F (F1)(Fig. 3). The remaining four adults (SM1, SM2, SM5, and SM6) differed in relatedness from SM412199F and were probably descended from a separate pair of anadromous adults that entered the Creek in early 1997. Analysis of the juvenile samples showed that three of the fish (SM15, SM16, and SM18) were of direct descent (F2) from SM412199F (F1). The remaining two juveniles (SM17 and SM19) had very divergent haplotypes, suggesting that more than one resident spawning pair bred successfully in DCC in 1999 (Nielsen and Sage 2001[4]) (Fig. 3). No useable material was obtained from SM14 and this sample was excluded from analysis.

The temperature probes were recovered in July 2002. All three probes successfully

[4]Nielsen J.L and G.K. Sage. 2001 Microsatellite and mtDNA analyses of San Mateo Creek trout, 2001. A progress report. 17 pp.

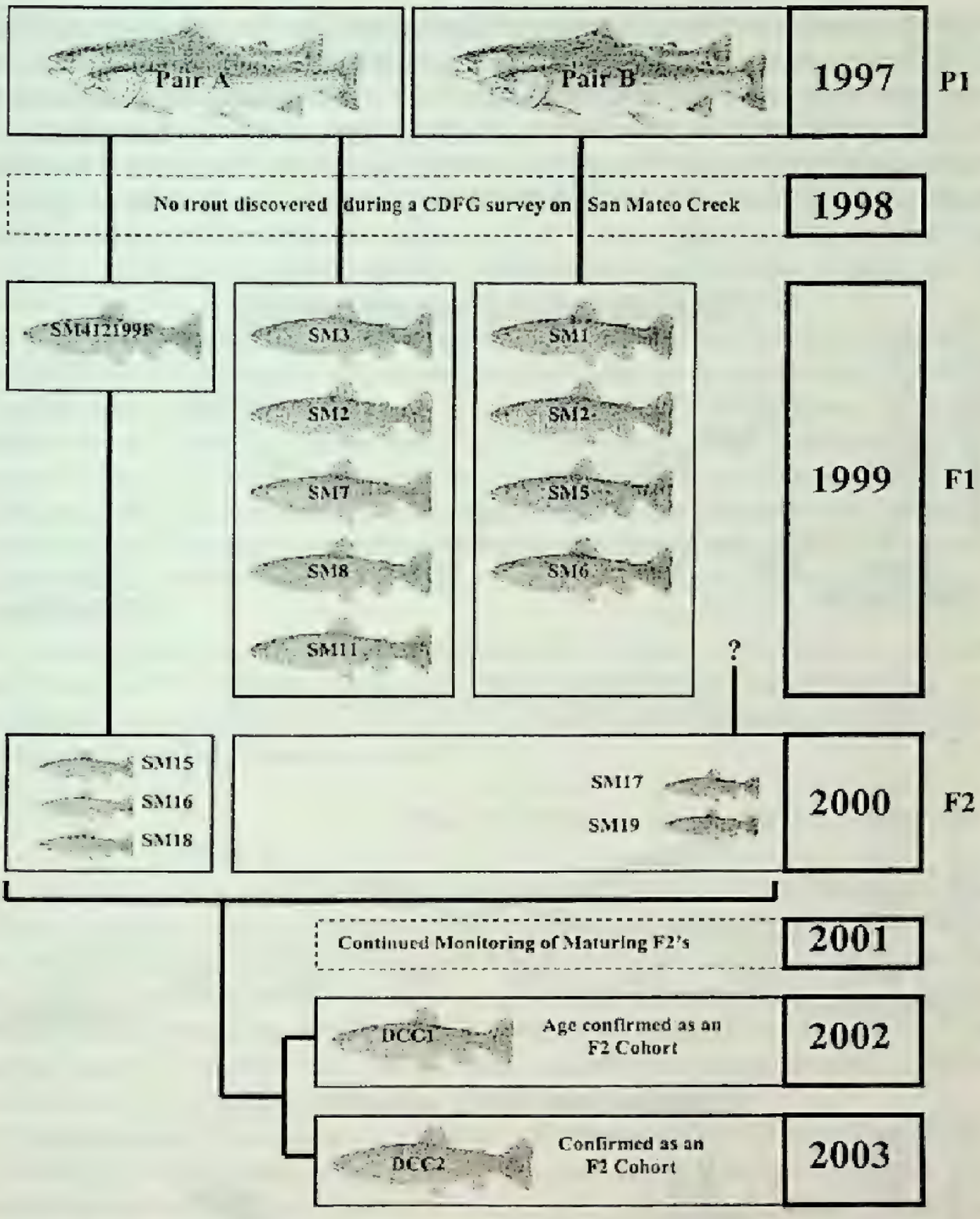


Figure 3. Chronology of trout presence on San Mateo Creek and Devil Canyon creek using genetic analysis, aging and observational data. 1997. Connected boxes indicate a confirmed genetic relationship. 1997:(P1) adults enter the San Mateo Creek drainage to spawn. 1998: No trout detected on San Mateo Creek during Department of Fish and Game Survey. 1999: A small population of 2 + year old, resident trout (F1) is detected on San Mateo Creek and Devil Canyon Creek. 2000: A small group of juvenile trout (F2) is discovered on Devil Canyon Creek in May and July. 2001: Continued monitoring of maturing F2 population on Devil Canyon Creek. 2002: The aging of a recovered adult on Devil Canyon Creek confirms individual is part of the 2000, F2 juveniles. 2003: The observation of a 3 ½ year old holdover fish on Devil Canyon Creek provides evidence of an uninterrupted fish presence on the San Mateo Creek drainage from 1997 to present.

collected data for the 11-month period of deployment. Two of the probes (DC13 and DC03) recorded very similar readings from August 2001 to March 2002. They recorded maximum temperatures of 21.8° C (DC13) and 23.1° C (DC03) and minimum temperatures of 7.8° C and 5.8° C, respectively (Fig. 4). The ranges within the first 7 months of deployment were almost identical between DC13 and DC03. For the last 4 months of deployment, DC13 recorded more consistent temperatures, with an increase from 10.1°

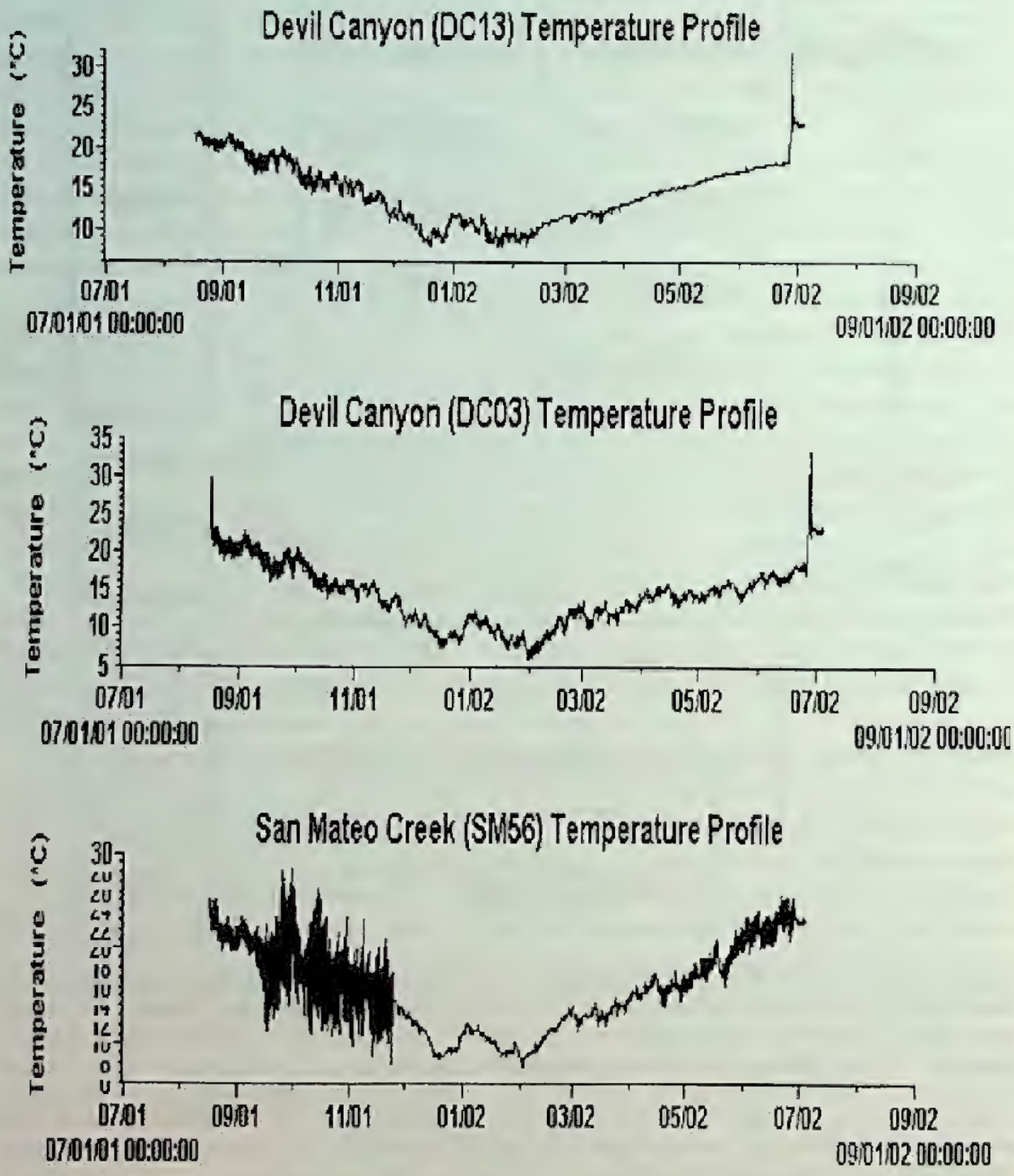


Figure 4. Temperature profiles for Devil Canyon Creek and San Mateo Creek from August 2001 to July 2002.

C to 18.1° C, with a daily range of +/- 0.9° C (Fig. 4). DC03 recorded an increase in temperature in that same period from 11.2° C to 18.9° C, with daily ranges of +/- 2.5° C (Fig. 4).

The SM56 temperature probe recorded severe temperature variations from August 2001 to December 2001, with readings from 28.4° C to 7.9° C, with some daily ranges shifting +/- 7.5° C (Fig. 4). Beginning in May 2002, SM56 recorded a steady increase in temperature, with daily ranges shifting +/- 1.4° C and topping out at time of retrieval at 25.8° C. This was in sharp contrast to the temperature maximums for DC13 and DC03 of 18.1° C and 18.9° C, respectively at time of probe retrieval (Fig. 4).

Age-at-growth calculations were performed on a single adult fish (DC1) collected in June 2002. The fish was recently expired and a complete work-up was performed. Both the right and left sagittal otoliths were removed and a single otolith was aged by four readers using a dissecting microscope (Fig. 2). The fish was determined to be 2+ years of age, and was a 273-mm TL female (gonad wt. 16.38 g), with a body weight of 213.5g. The eggs of the fish were partially hydrated upon removal and appeared of high quality. Both lobes of the ovaries were ruptured and the stomach was empty. Genetic material was collected, but has yet to be analyzed. No parasites were observed, internally or externally.

A single adult, holdover trout (DC2) was observed on the confluence as recently as December 2003. This specimen was determined to be a F2 holdover fish due to its size, coloration, and location on the creek. This observation established an uninterrupted fish presence on the drainage since 1997.

## DISCUSSION

Almost immediately following the discovery of southern steelhead on San Mateo Creek, Department surveys began to record a decline in trout numbers towards the end of 1999. Observations of trout in an apparent emaciated condition strongly suggested that resource competition with exotics was impacting trout survival. Additionally, the recorded water temperature within SMC during trout presence strongly favored the exotic species over the trout, and probably contributed greatly to their eventual extirpation.

The discovery of juvenile trout in the lower reaches of DCC in May and July of 2000 became a pivotal observation during the survey period. This illustrated that even with infrequent and intermittent steelhead trout migration into the drainage, successful resident (F1) reproduction could result. These observations also confirmed that adequate resident spawning habitat does exist in DCC, and given the proper conditions, resident spawning could occur. This tributary is completely free of exotic fish species and holds promising trout habitat only a short distance from the SMC. Additionally, temperature stratified pools, deep enough and cold enough to support trout year-round are readily available in the drainage.

The genetic analysis was crucial in not only establishing the lineage of the sampled trout within the drainage, but also proving a connection between the trout located in both drainages. The trout first analyzed (SM412199F) was collected in DCC and the

confirmed cohorts (SM4, SM7, SM8, and SM11) were all collected in SMC, indicating either upstream or downstream migration of the residents. Since we observed trout in declining health, co-occurring with exotics in SMC, the survey observations provide strong evidence that downstream migration of the residents from DCC to SMC likely occurred.

The positive identification of direct descendents (Samples SM15, SM16, and SM18) from the previously sampled SM412199F indicates that the trout population size on the drainage is extremely small. This is also the first evidence supporting successful reproduction and survival of F2 offspring from this F1 cohort (SM412199F)(Table 1).

Since the two remaining juvenile samples were shown to be derived from a yet unsampled resident pair, we concluded that additional, undocumented resident fish may still occupy the drainage, further elevating population numbers.

The temperature data obtained from the probes clearly shows that Devil Canyon maintains a more consistent and preferential temperature profile for southern steelhead/rainbow trout survival than portions of San Mateo Creek (Fig. 4). Southern races of steelhead have evolved a much higher tolerance to high temperatures and low dissolved oxygen levels than is generally thought suitable for juvenile salmonids (Greenwood 1988), and the preferred water temperature for various life stages of steelhead is well documented (Bovee 1978: Reiser and Bjornn 1979[5]: Bell 1986). While this adaptation to adverse water conditions may assist in southern steelhead survival, temperature stability and reduced daily range shifts would be more favorable for trout occupying this drainage.

Both SMC and DCC exhibit ideal southern steelhead life history temperatures (McEwan and Jackson 1996[6]) for a portion of the year, but, during the summer, sections of the drainage experience surface water temperatures in excess of 26.7° C, well above suitable salmonid preference. Temperature stratification occurs throughout the SMC drainage and has been documented in pools occupied by trout. However, during dryer years, pool stratification and survivable temperatures for salmonids becomes limited. The probe data suggests that the over-summering habitat available in Devil Canyon provides a more stable temperature environment for steelhead sustainability and survival.

The collection of the single dead individual (DC1) on DCC during the summer of 2002 enabled us to not only confirm the age of the individuals that were currently being monitored on DCC, but also allowed us to examine the reproductive status of the collected specimen. The fish was 2 + years of age, confirming that the like-sized individuals occupying DCC were indeed cohorts of the 2000 juvenile group discovered in the lower portion of the drainage in May and July of that year. Upon dissection, no anomalies were discovered. The weight of the two ovaries made up over 7.5% of the

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[5]Reiser, D.W., and T.C. Bjornn. 1979. Habitat requirements of anadromous salmonids. USDA, Forest Service, Pacific Northwest Forest and Range Experimentation Station, Portland, Oregon, General Technical Report PNW-96, 54 pp.

[6]McEwan, D. and T.A. Jackson 1996. Steelhead restoration and management plan for California. California Department of Fish and Game, Sacramento, CA.

body weight of the fish and the eggs looked to be partially hydrated in life. This indicated that this fish could have participated in spawning activity during spring 2002, given the opportunity. There was no outward indication of why the fish died. However, the pool in which the fish was collected, had a water temperature of 25°C at the time of collection, and it is likely the fish succumbed to depleted dissolved oxygen levels in the shallow, overheated pool.

The additional genetic, age, and monitoring information collected during this survey period clarifies the sequence of events for the population of steelhead/rainbow trout on the San Mateo Drainage (Fig. 3). Back calculations of the individuals aged in 1999 confirm that the anadromous adults (P1) entered the drainage in the early spring 1997 to spawn. Genetic analysis of samples SM1, SM2, SM5, and SM6 confirm the presence of an additional anadromous pair in the drainage in 1997. No fish were detected during a Department survey on San Mateo Creek in 1998. In the following year, a small population of trout (F1) was discovered occupying San Mateo Creek. All trout observed were of a single age class (2+ years), indicating they were the offspring of a single, reproductive event. In 2000, juvenile trout (F2) were discovered in the lower reaches of Devil Canyon Creek. Genetic analysis linked these fish to the F1 population, some directly descended from the first fish analyzed (SM412199F). The presence of sub-adults on the drainage in November 2000 suggests that they were maturing F2's. Age analysis of a single adult (DC1) collected in June 2002 confirms that a few of the adult fish currently occupying DCC are mature F2's (Fig. 3). Surveys conducted on SMC and DCC in 2001 and 2002 revealed no new recruitment during that time. The discovery of a 3.5-year-old holdover fish (DC2) in DCC in December 2003 provides evidence that there has been an uninterrupted trout presence on the drainage since the anadromous adults entered SMC during the spring of 1997 to spawn.

While the timeline of trout presence on the drainage can be firmly established with the evidence gathered during the study period, some question can be raised on where the original steelhead adults spawned in 1997. Surveys conducted in 1995, 1996, and 1997 by the United States Fish and Wildlife Service (USFWS) and a Department survey conducted in 1998 failed to detect trout on SMC. If anadromous adults entered the drainage and spawned in San Mateo Creek in 1997, young-of-the-year individuals should have been detected in fall 1997 by USFWS personnel and yearling individuals should have been detected in 1998 by Department personnel. All surveys included the stream portion from the USGS gauging station to above the DCC confluence, but did not include DCC. Since the age of the 1999 individuals has been confirmed at 2+ years of age and the fish were all cohorts, they had to be present on the drainage during the 1997 and 1998 surveys. Since the upstream migration opportunities from SMC to DCC have been extremely limited since 1999, and trout were absent in SMC when those opportunities existed, we can conclude that all trout observed during the original discovery in 1999, migrated downstream from DCC into SMC. We can also conclude that the individuals discovered in 1999 were attempting to emigrate out of DCC at the time of discovery, as several of them were observed in the silvery, smoltification stage when captured.

The impacts of exotic fish competition and predation on native fish is well

documented and while direct competition is difficult to quantify, the observation of resident trout on SMC in an unhealthy state co-occurring with nonnatives suggests that trout survival is severally hindered by the constant presence of exotic fish species. Exotic fish species (largemouth bass, green sunfish, bluegill and black bullhead) have been present in great numbers within the San Mateo Creek drainage for many years (USFWS status report, 1998; Woelfel 1991[7]) and have been shown to have a strong competitive edge over resident trout. Green sunfish have been found to feed on juvenile trout and out-compete adult steelhead for benthic food (Swift 1975[8]; Greenwood 1988). Largemouth bass take over as top predator in the habitat they occupy and can directly predate steelhead (Stouder et al. 1997).

Other studies have found that competition between introduced, warm water exotics and juvenile steelhead, when water conditions are warm, favored the exotics (Reeves 1985[9]). Later studies inferred that competition between exotic species and steelhead was limiting production of steelhead in some Umpqua River, OR.tributaries (Fontaine 1988[10]). While exotic fish presence may have played some role in steelhead trout decline on SMC, the combination of a higher water temperature and limited water supply likely contributed.

A portion of the information collected during this 4-year monitoring program provided NMFS with enough data to re-evaluate the ESU geographic unit for the endangered southern steelhead trout established in 1997. On May 2002, NMFS published their final rule for the range extension for endangered steelhead in southern California. Citing the extensive monitoring information collected by the Department and the new genetic analysis conducted by Dr. Jennifer Nielsen, the NMFS concluded that the *O. mykiss* population in San Mateo Creek is part of the listed southern California steelhead population and worthy of protection. Also citing the San Mateo Creek population, NMFS decided not to delineate a specific stream as the southern boundary as it had in 1997. Instead, the final rule states that the listed *O. mykiss* population extends from the Santa Maria River to the southern extent of its range. This provides additional protection for future discoveries within the entire endangered southern steelhead range. The individuals currently occupying the San Mateo Creek drainage represent the southern most documented population of *O. mykiss*.

It is clear that two definitive processes combine to influence the survival of the southern steelhead trout population on the San Mateo Drainage; water availability and

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[7]Woelfel, D. 1991. The restoration of San Mateo Creek: A feasibility study for a southern California steelhead fishery. MS. Thesis, California State University, Fullerton

[8]Swift, C.C., A.W. Wells, and J.S. Diana. 1975. Survey of the freshwater fishes and their habitats in the coastal drainages of southern California. Natural History Museum of Loa Angeles County. Final Report to California Department of Fish and Game, Inland Fisheries Division. 364 pp.

[9]Reeves, G. 1985. Competition between redside shiners and steelhead trout juveniles in the Umpqua River. Dissertation, Oregon State University, Corvalis, Oregon

[10]Fontaine, B. 1988. An assessment of habitat enhancement measures and factors limiting production of steelhead trout in tributaries to the Umpqua River. M.S. Thesis, Oregon State University, Corvalis, Ore.

the presence or absence of exotic fish species. For the last 60 years, Marine base Camp Pendleton has been pumping ground water from the aquifer beneath the SMC drainage. While stream conditions are currently capable of supporting a steelhead population, alternatives to reduce or eliminate this pressure on the resource can only benefit the trout. Additional efforts are needed to seek an acceptable and effective method for controlling exotic fish species on the drainage. Regardless of the methods employed, this undertaking cannot be successful in the long-term unless re-infestation from the source is controlled, if the source still exists. Currently, the populations of exotic fish species within the drainage are self-sustaining and annual repopulation of the creek occur from adults within the creek.

Even though the San Mateo drainage steelhead/rainbow trout have extremely low population numbers based on Department surveys, their continued survival in extremely harsh conditions makes their preservation even more important. All juvenile coastal rainbow trout have the potential to emigrate from their coastal stream and become steelhead irrespective of their parentage, and therefore are extremely important to the persistence of the population (Behnke 1992). This polymorphic population structure allows persistence in an environment that is frequently suboptimal and populations of this type may be necessary for the long-term persistence of the population (Johnson 1985, Titus and Mosegaard 1992, Northcote 1997). Often species occupying the margins of their range benefit from a polymorphic life history where conditions may be unstable. This life-history plasticity, in a drainage that experiences unreliable stream flow for immigrating adults and emigrating smolts, and a constant exotic presence, is undoubtedly the reason southern steelhead/rainbow trout continue to occupy the San Mateo Creek drainage.

## ACKNOWLEDGMENTS

Dr. J. Nielsen provided essential comments on the genetic portion of this manuscript. I am thankful to all the reviewers that assisted in the editing of the text and to C. Miller for providing assistance on the figures.

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Received: 13 September 2002
Accepted: 3 September 2003

*California Fish and Game* 90(3):155-156 2004

# RECORD OF NESTING BY FULVOUS WHISTLING-DUCKS, SOUTHERN SAN JOAQUIN VALLEY, CALIFORNIA

GREG GERSTENBERG
California Department of Fish and Game
1551 Greenwood Way, Los Banos, CA 93635
ggerstenberg@dfg.ca.gov

CARL D. REY[1]
United States Fish and Wildlife Service
Kern National Wildlife Refuge
P.O. Box 670, Delano, CA 93216

Occurrence of nesting by fulvous whistling-ducks, *Dendrocygna bicolor*, in the Southern San Joaquin Valley (SSJV) was well documented between 1898 and 1922 (Shields 1899, Bryant 1912, Bryant 1914, Dickey and Van Rossem 1923). Grinnell and Miller (1944) described the historical geographic range of this species as including parts of the San Joaquin Valley (SJV). The most recent documentation of nesting fulvous whistling-ducks in the SJV involved numerous sightings in 1922 at Buena Vista Lake (Dickey and Van Rossem 1923), and a small colony in Fresno County in the early 1960's (Bellrose 1980). Department personnel working the Mendota Wildlife Area from 1957-1960 saw many adult fulvous whistling-ducks through the summer months, but did not observe any ducklings or nests (R.H. Brueggemann, personal communication).

Reclamation of Tulare, Kern, and Buena Vista lakes for agricultural purposes virtually eliminated the vast complex of perennial marshes which historically provided extensive areas of nesting habitat in the SSJV (U.S. Fish and Wildlife Service[2] 1978). Although these ancient lakebeds have flooded periodically during years of above average runoff, documented nesting activity by fulvous whistling-ducks in these lakes has not been recorded since their reclamation. In the spring of 1983, seasonal precipitation was approximately 188% of average and as a result of abnormally high runoff, there were up to 48,340 ha of surface water in the Tulare Lake Basin during the summer months (U.S. Fish and Wildlife Service[3] 1983).

Two broods of fulvous whistling-ducks were observed by the authors during the summer of 1983 in the SSJV. On 24 June 1983, 4 adult and 5 Class Ia (after Gollop and Marshall 1954) juvenile fulvous whistling-ducks were observed by C. Rey in the southwest quarter of Unit #7 on Kern National Wildlife Refuge (Section 10, T25S, R22E, Mount Diablo Base and Meridian) Kern County. The habitat was dominated by salt cedar, *Tamarix pentandra*, intermixed with open water channels and mud flats. Two

[1]Current Address: c/o Hot Springs Ranch, Route 1, Box 2015, Corral, ID 83322.

[2]U.S. Fish and Wildlife Service. 1978. Concept plan for waterfowl wintering habitat preservation - Central Valley, California. U. S. Fish and Wildlife Service, Portland, OR. 116 pp.

[3]U. S. Fish and Wildlife Service. 1983. Kern National Wildlife Refuge Complex Annual Narrative.

adults observed on the water with five ducklings subsequently flushed by the approaching boat, flew to a location some 75 m distant, and landed in an open water channel near two other adults.

On 26 July 1983, the second brood was observed by G. Gerstenberg. One adult and 3 Class Ib juvenile fulvous whistling-ducks were sighted on the water near the edge of a pre-irrigated barley, *Hordeum vulgare*, field (Section 27, T24S, R21E, MDB&M, Kings County). Pre-irrigation consisted of a section of land where barley had been harvested and flooded to a depth of 0.2 - 0.5 m. The single adult feigned injury as it attempted to lure us away from the ducklings. A second sighting of three ducklings at the same location occurred on 9 August when two adults were observed in the immediate vicinity of three sick Class IIa ducklings. The ducklings were on the bank and unable to swim or walk. The ducklings were removed as part of a routine avian botulism control program and held at the Kern National Wildlife Refuge waterfowl treatment facility. The three ducklings died within 2 days, presumably from botulism. These are presently held as specimens by Humboldt State University (HSU numbers 5422 and 5466) and Reedley College. On 28 July, seven adult-sized fulvous whistling-ducks were observed by G. Gerstenberg flying in this same location. Discussions with agency personnel working the SJV since 1983 have failed to reveal any additional observations of nesting or ducklings.

## ACKNOWLEDGMENTS

We thank Dr. R. Gambs, Dr. R. Golightly, R. Goss, J. Houk, Dr. E. Johnson, and G. Kramer for their comments and suggestions on early drafts.

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Received: 16 August 2002
Accepted: 8 July 2003

*California Fish and Game* 90(3):157-159 2004

# FIRST RECORD OF THE CAPE CIGARFISH, *CUBICEPS CAPENSIS* (FAMILY NOMEIDAE), FROM CALIFORNIA WATERS

JOHN E. McCOSKER[1], SHANE ANDERSON[2], JOHN RICHARDS[3]
and MILTON LOVE[3]
[1]California Academy of Sciences
San Francisco, CA 94118
jmccosker@calacademy.org
[2]University of California, Santa Barbara
Santa Barbara, CA 93106
[3]UCSB Marine Science Institute
Santa Barbara, CA 93106

While retrieving a surface gill net they had set for sharks and swordfish from the F/V *Diane Susan* at 35°08'N, 122°24'W (approximately 145 km WSW of Morro Bay, San Luis Obispo County, California), Tom and Diane Roff observed a cigarfish trapped in the net. The fish was caught on 5 September 2003 over 1800 fms (3291 m) depth; surface temperature was 18.6°C. The water was blue with very few fish on the fish finder; the drift was southerly over a distance of approximately 18 km, and the clean net (no slime or jellyfish) was recovered with only the cigarfish, an albacore, *Thunnus alalunga*, and a shortfin mako shark, *Isurus oxyrinchus*.

The specimen, in nearly perfect condition, was placed on ice and given to Richards. Anderson photographed it (Fig. 1) and Love removed its right sagitta. Fresh tissue was removed and preserved in 95% ethanol and the tissue and the specimen (CAS 218171) are now deposited in the Department of Ichthyology of the California Academy of Sciences in San Francisco. Using Agafonova's (1994) revision of the genus we identified it as *Cubiceps capensis*, the Cape cigarfish, a nomeid known from the southern and northern subtropical Pacific, Indian, and Atlantic oceans, but previously

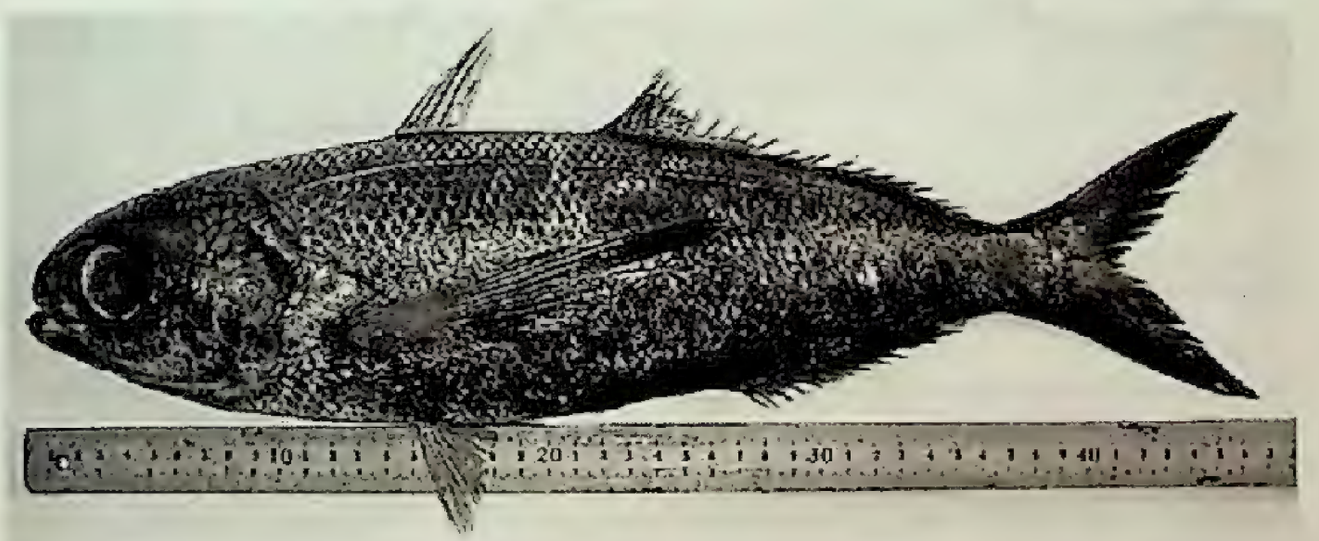

Figure 1. *Cubiceps capensis* (CAS 218171, 374 mm SL) from off California, photographed soon after capture.

unreported from California waters. The specimen is a mature male with firm gonads, 374 mm SL (460 mm TL), and has the following characteristics: D XI,I 22; A III,22; P 21; 67 lateral line scales; 31 vertebrae; a minute uniserial row of teeth on the vomer and tongue; snout rounded; profile of back and belly moderately convex, the greatest body depth at the dorsal origin; eye fairly large, 4.2 in head; upper jaw 3.7 in head; snout tip and preorbital scaleless; and the coloration in life of its fins, back, sides, and head was grayish-brown to black, except for its caudal fin and belly, which were nearly black. Agafonova (1994: 136) reported that the otolith of *C. capensis* has not been described; we herein illustrate (Fig. 2) and compare its sagitta to those of some of its congeners. The otolith is relatively large (~5 % SL) for that of a *Cubiceps* and most comparable in appearance to that of the *C. squamiceps* drawn by Agafonova (1994: fig. 3d, but note that he has illustrated the left sagitta).

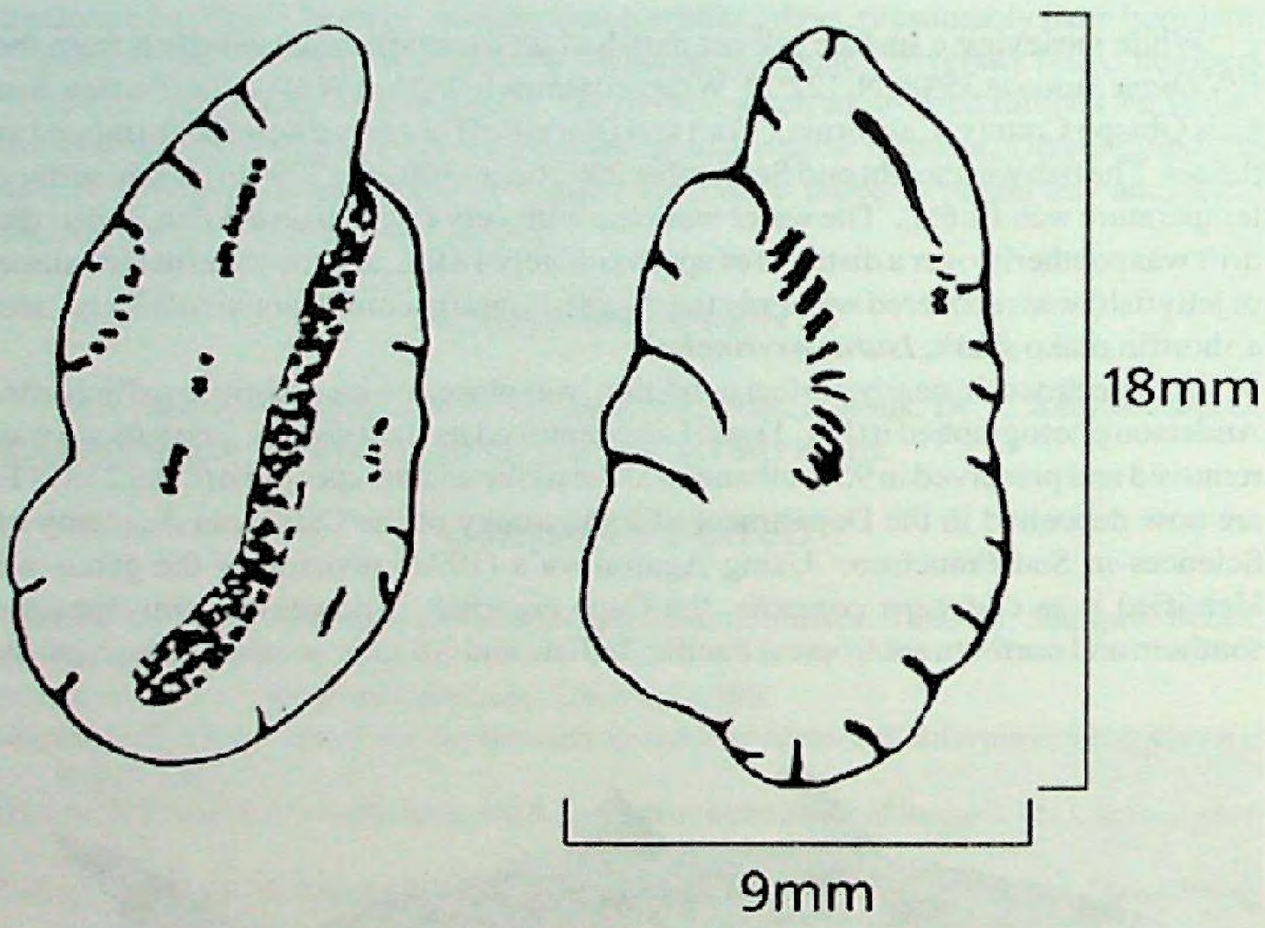


Figure 2. Inner (left) and outer (right) surfaces of the right sagitta of *Cubiceps capensis* (CAS 218171, 374 mm SL).

This is the third record of a *Cubiceps* from California, previously known only from a specimen of *C. paradoxus* caught by hook and line off Portuguese Bend, Los Angeles County, in 1954 (Butler 1979), and another that was gaffed in Los Angeles Harbor in 1986 (Ellis et al. 1988). The 1954 specimen was identified by Fitch and Lavenberg (1968: 118) as *C. gracilis*, but was later described as the holotype of *C. paradoxus* by Butler (1979). Although nearly identical in coloration and general appearance, *C. paradoxus*

differs from *C. capensis* in having a more tapered snout, a more slender body, a smaller eye (5.1-6.1 in head length), a shorter upper jaw (4.2-4.4 in head length), and in lacking teeth on its tongue.

## ACKNOWLEDGMENTS

We thank: T. and D. Roff for their capture and careful handling of the specimen; J. Fong of the California Academy of Sciences (CAS) for preparation of the radiograph; and T. Iwamoto (CAS) for assistance with this manuscript.

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Received: 1 December 2003
Accepted: 4 December 2004

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